Thermo Scientific Pierce Protein Purification Technical Handbook

Version 2

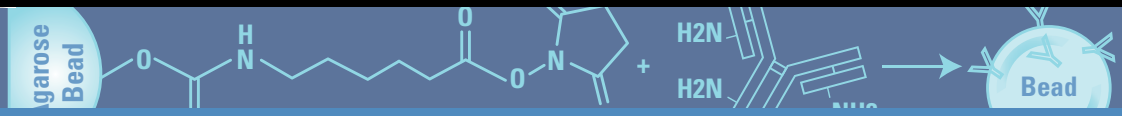

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Table of Contents

Introduction to Protein Purification

General Protein Purification Techniques

Protein purification is essential for a host of biochemical applications. However, with thousands of proteins each displaying unique characteristics, it is important to develop a strategy for purification that delivers the correct yield, purity and activity needed for downstream applications. For low resolution/high yield protein purification, methods such as fractional precipitation using salts such as ammonium sulfate exist. For applications requiring the highest purity and relatively small amounts of protein, affinity purification techniques can be chosen to selectively extract a target protein from the complex mixture of proteins found in cell or tissue extracts. Table 1 summarizes a few general strategies.

Each of these protein purification techniques requires specific buffers (mobile phase), chromatography resins (solid phase) and column accessories. These three components can be used in a variety of different configurations, based on the scale of purification and available equipment. Common formats include:

Batch purification

Mixing the mobile and stationary phases in a conical tube and separating the two via centrifugation. Batch method purification can be performed at any scale. However, it is most commonly reserved for microcentrifuge tube scale purifications involving 10-200µL of resin. In batch method purification, wash and elution fractions are separated from the resin after centrifuging to pellet the resin beads. The liquid cannot be removed completely because some of it is contained within the volume of porous bead pellet. Consequently, a portion of each fraction about equal to the volume of resin used is left behind in the pellet, making washes and elution somewhat inefficient.

Gravity flow chromatography

Passively adding the mobile phase to packed columns, without mechanically increasing the flow rate. Gravity flow commonly uses 1mL- to 5mL-packed columns set-up on a bench or in a chromatography refrigerator. Larger columns can be packed to support larger scale protein purification. However, the weight of the resin in the column must be considered to prevent damage to resin at the bottom of the column. Gravity flow allows for extended binding, washing and elution times, which is ideal for samples with low binding affinity.

Table 1. Summary of protein purification techniques.

Resolution/ purity	Technique	Protein Yield	Description
Low	Ammonium sulfate precipitation	High	Fractional precipitation of proteins based on their solubility in salt solutions of varying saturation
	Hydrophobic interaction chromatography	High	Separation of proteins based in their surface hydrophobicity
	Size exclusion chromatography (SEC)	Medium	Separation of proteins based on molecular size
Medium	Ion exchange chromatography	Medium	Separation based on protein charge at a particular pH
	Gel electrophoresis	Medium	Two-step separation of protein based on size using polyacrylimide gel electrophoresis (SDS-PAGE) and charge using isoelectric focusing. Note: This is a denaturing method that results in a complete loss of protein activity.
	Proteome fractionation	Medium	Enrichment of classes of proteins, such as: • phosphoproteins using immobilized metal affinity chromatography (IMAC) • glycoproteins using immobilized lectins • nitrosylated or palmitylated proteins using the biotin switch assay • organelle-specific proteomes using cellular fractionation techniques
High	Affinity purification	Low	Selective purification using affinity tags attached to the target gene, such as polyhistidine (6xHis), glutathione S-transferase (GST) or maltose binding protein (MBP)
	Immunoprecipitation	Lowest	Antibody-based extraction of a single protein species, typically from cell or tissue lysatesh

Introduction to Protein Purification

Spin cup purification

Separating the mobile and stationary phases using centrifugation in packed spin tubes with filters that retain resin in column. Spin purification can also be performed with spin plates, where each well of a 96-well microtiter plate has a filter base and is packed with the appropriate chromotagraphy resin. The spin cup purification method provides improved efficiency of wash and elution steps compared to the batch method. Centrifugation separates the liquid fraction by pulling it thoroughly from the resin, which is retained within the spin cup apparatus. Spin cup purification is most appropriate when 50-300µL of immobilized ligand resin is used.

Magnetic purification

Isolating the stationary phase using magnets. Magnetic beads are commonly iron oxide particles which are coated with a ligand to purify a protein target. An example of this is a magnetic particle coated with reduced glutathione (GSH) used to purify GST-tagged recombinant proteins (Product # 88821). Advantages of using magnetic beads include easy handling, minimal loss of resin from pipetting and compatibility with high throughout automated systems such as the Thermo Scientific KingFisher 96 instrument.

Fast protein liquid chromotagraphy (FPLC)

Using chromatography cartridges pre-packed with the stationary phase and a series of pumps and UV detectors to move and monitor the mobile phase. The advantages of using FPLC include reduced purification times and the ability to improve resolution by linking multiple columns in tandem for greater separation.

Affinity Purification

Various methods are used to enrich or purify a target protein from other proteins and components in a crude cell lysate or other sample. The most powerful of these methods is affinity purification, also called affinity chromatography, whereby the protein of interest is purified using its specific binding properties to an immobilized ligand.

Affinity purification makes use of specific binding that occurs between molecules and is used extensively for the isolation of biological molecules. A single pass through an affinity column can achieve a 1,000- to 10,000-fold purification of a ligand from a crude mixture. From a single affinity purification step, it is possible to isolate a compound in a form pure enough to obtain a single band upon SDS-PAGE analysis. We offer a number of immobilized protein or ligand products for affinity purification of antibodies, fusion-tagged proteins, biotinylated proteins and other proteins for which an affinity ligand is available.

In affinity purification, a ligand is immobilized to a solid support. Once immobilized, it specifically binds its partner under mild buffer conditions (often physiological conditions such as phosphate buffered saline). After binding to the partner molecule, the support is washed with additional buffer to remove unbound components of the sample. An elution buffer is added, disrupting the interaction between the ligand and its binding partner by pH extremes (low or high), high salt, detergents, chaotrophic agents or the removal of some factor required for the pair to bind. Once released, the binding partner can be recovered from the support using additional elution buffer. The elution buffer can then be exchanged by dialysis or desalting into a more suitable buffer for storage or downstream analysis.

Activated affinity support products and kits enable a researcher to immobilize nearly any type of ligand to purify its binding partner(s). For example, if a peptide antigen is used to immunize animals and produce antibodies, the same peptide can be immobilized to a gel support and used to affinity-purify the specific antibody from animal serum. Alternatively, if a specific antibody is available against a particular protein of interest, it can be immobilized to a support and used to affinity-purify the protein from crude cell lysate. Purification with respect to nearly any binding interaction can be made by this approach.

Affinity purification products using either immobilized ligands or activated affinity support chemistries are available for use in several different formats. Most commonly, porous beaded gel supports are used for gravity-column, spin-column or batch-scale purification procedures. Coated microplates are available for high-throughput screening applications, and magnetic particles are especially useful for automated protein purification.

Proteins and other macromolecules of interest can be purified from crude extracts or other complex mixtures using a variety of methods. Precipitation is perhaps the simplest method for separating one type of macromolecule from another. For example, nucleic acids can be precipitated and thereby purified from undesired molecules in solution using ethanol, and proteins can be selectively precipitated in the presence of ammonium sulfate.

Most purification methods involve some form of chromatography whereby molecules in solution (mobile phase) are separated based on differences in chemical or physical interaction with a stationary material (solid phase). Gel filtration (also called desalting, size exclusion chromatography or SEC) uses a porous gel material to separate molecules based on size; large molecules are excluded from the internal spaces of the gel material while small molecules enter the resin pores, resulting in a longer path through the column. In ion exchange chromatography, molecules are separated according to the strength of their overall ionic interaction with a solid-phase material. By manipulating buffer conditions, molecules of greater or lesser ionic character can be bound to or dissociated from the solid-phase material.

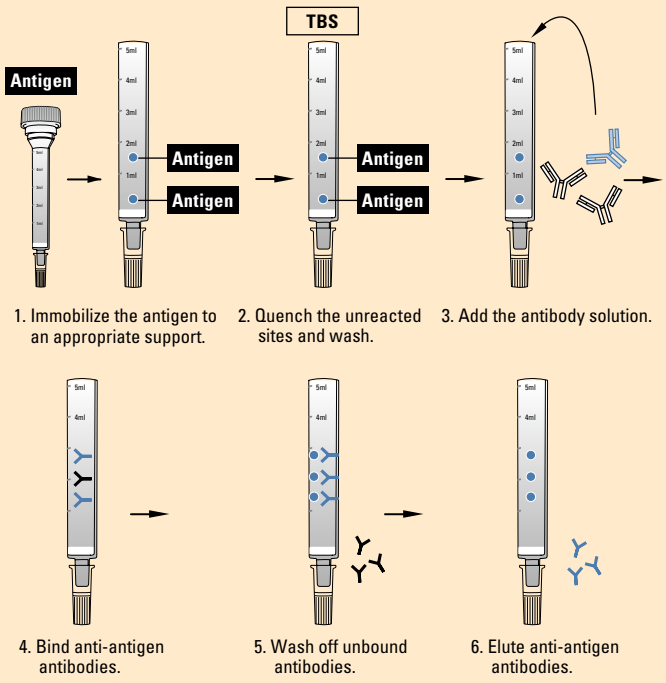
In contrast, affinity chromatography or affinity purification makes use of specific binding interactions between molecules. A particular ligand is chemically immobilized or "coupled" to a solid support so that when a complex mixture is passed over the column, only those molecules having specific binding affinity to the ligand are purified. Affinity purification generally involves the following steps:

1. Incubate crude sample with the immobilized ligand support material to allow the target molecule in the sample to bind to the immobilized ligand.
2. Wash away unbound sample components from solid support.
3. Elute (dissociate and recover) the target molecule from the immobilized ligand by altering the buffer conditions so that the binding interaction no longer occurs.

Ligands that bind to general classes of proteins (e.g., Protein A for antibodies) or commonly used fusion protein tags (e.g., glutathione for GST-tagged proteins) are available in pre-immobilized forms ready to use for affinity purification. Alternatively, more specialized ligands such as specific antibodies or antigens of interest can be immobilized using one of several activated affinity supports; for example, a peptide antigen can be immobilized to a support and used to purify antibodies that recognize the peptide.

Most commonly, ligands are immobilized or "coupled" directly to solid support material by formation of covalent chemical bonds between particular functional groups on the ligand (e.g., primary amines, sulfhydryls, carboxylic acids, aldehydes) and reactive groups on the support. However, other coupling approaches are also possible. In the Thermo Scientific GST Orientation Kit (Product # 78201), for example, a GST-tagged fusion protein is first bound to an immobilized glutathione support by affinity interaction with the GST tag and then chemically crosslinked to the support. The immobilized GST-tagged fusion protein can then be used to affinity-purify its binding partner(s). Likewise, the Thermo Scientific Pierce Crosslink Immunoprecipitation Kits (Product # 26147) and Thermo Scientific IgG Orientation Kits (Product # 44990) involve binding and subsequent crosslinking of an antibody to immobilized Protein A, A/G.

Historically, researchers have used affinity purification primarily to purify individual molecules of interest. Increasingly, proteomics research focuses on determination of disease states, cell differentiation, normal physiological functions and drug discovery involving interaction and expression of multiple molecules rather than individual targets. Consequently, the use of affinity methods has expanded to purification of native molecular complexes and forms the basis for co-immunoprecipitation (co-IP) and "pull-down" assays involving protein:protein interactions.



Typical antibody purification using an immobilized antigen column.

Affinity Purification Supports

Affinity purification involves the separation of molecules in solution (mobile phase) based on differences in binding interaction with a ligand that is immobilized to a stationary material (solid phase). The solid phase in affinity purification is a support or matrix material that a biospecific ligand may be covalently attached. Typically, the material to be used as an affinity matrix is insoluble in the system in which the target molecule is found. Usually, but not always, the insoluble matrix is a solid. Hundreds of substances have been described and employed as affinity matrices.

Useful affinity supports are those with a high surface area to volume ratio, chemical groups that are easily modified for covalent attachment of ligands, minimal nonspecific binding properties, good flow characteristics, and mechanical and chemical stability. When choosing an affinity support or matrix for any separation, the most important question to answer is whether a reliable commercial source exists for the desired matrix material in the quantities required. Fortunately, we offer a wide range of practical and efficient matrices in volumes ranging from 1mL to much larger bulk quantities.

Porous Beaded Resins

Porous beaded supports generally provide the most useful properties for affinity purification of proteins. We offer affinity purification products in two main porous gel support formats: crosslinked beaded agarose and Thermo Scientific UltraLink Biosupport. The various features of these two supports are listed in the accompanying table. Agarose is good for routine applications but crushes easily, making it suitable for gravity-flow column or small-scale batch procedures using low-speed centrifugation. UltraLink Biosupport is incompressible and can be used in high-pressure applications with a peristaltic pump or other liquid chromatography system. In addition, UltraLink® Supports display extremely low nonspecific binding characteristics because they are polyacrylamide-based. Both supports perform well in typical gravity-flow and spin column purification and immunoprecipitation procedures.

Magnetic Particles

When a matrix is required for affinity purification of cells within a population, we recommend Thermo Scientific MagnaBind Beads. Magnetic affinity separation is a convenient method for isolating antibodies, antigens, lectins, enzymes, nucleic acids and cells while retaining biological activity. Samples containing the molecule of interest are incubated with beads that are derivatized with an antibody or other binding partner. A rare earth magnet is used to pull the MagnaBind™ Beads out of solution and onto a surface. The buffer can be carefully removed, containing any non-bound molecules or cells.

MagnaBind Beads consist of a silanized surface over an iron oxide core (see Table 2). The silanized surface has been derivatized to contain active groups, such as carboxylic acids or primary amines, or specific affinity molecules such as streptavidin; Protein A; Protein G; or goat anti-mouse, anti-rabbit or anti-rat IgG. Due to the nature of the MagnaBind Beads, strong elution conditions are not recommended with these products. See page 80 for a complete listing of MagnaBind Supports.

Physical properties of porous gel supports.

Support	4% Agarose (crosslinked beaded agarose)	6% Agarose (crosslinked beaded agarose)	Thermo Scientific UltraLink Biosupport (co-polymer of crosslinked bis-acrylamide and azlactone)
Bead	45–165µm	45–165µm	50–80µm
Exclusion Limit	20,000,000 daltons	4,000,000 daltons	2,000,000 daltons (1,000 Å pore size)
Durability	Crushes under pressure	Crushes under pressure	Sturdy (>100 psi, 6.9 bar)*
Types of Chromatography	Gravity and small spin columns	Gravity and small spin columns	FPLC Systems, medium pressure, gravity flow
Coupling Capacity	Medium	Medium	High
pH range	3–11	3–11	3–11
Form	Preswollen	Preswollen	Dry or Preswollen

* ***Note****: The indicated maximum pressure of 100 psi refers to the maximum pressure drop across the gel bed that the support can withstand. It does not necessarily refer to the indicated system pressure shown on a liquid chromatography apparatus because the system pressure may not actually be measuring the pressure drop across the column. Typical system pressures are usually much higher due to pumping through small I.D. tubing, auto-samplers, detectors, etc. When packed into a 3mm ID x 14cm height glass column, these exclusive supports have been run to approximately 650 psi (system pressure) with no visual compression of the gel or adverse effects on chromatography. These columns can be run at linear flow rates or 85–3,000cm/hour with excellent separation characteristics.*

Table 2. Characteristics of underivatized Thermo Scientific MagnaBind Beads.

Composition	Silanized iron oxide
Magnetization	25–35EMU/g
Type of Magnetization	Superparamagnetic (no magnetic memory)
Surface Area	>100m^2/g
Settling Rate	4% in 30 minutes
Effective Density	2.5g/mL
Number of Beads	1 x 10^8 beads/mg
pH Stability	Aqueous solution, above pH 4.0
Concentration	5mg/mL

Note: *To establish a microbe-free preparation, MagnaBind Beads can be washed with antibiotic medium or γ-irradiated.*

Microplates

Polystyrene microplates are another type of matrix commonly used for immobilization of proteins. Proteins passively adsorb to the polystyrene surface through hydrophobic interactions. Generally, this adsorption of proteins onto the polystyrene surface occurs best in carbonate/bicarbonate buffer at an alkaline pH (9.0–9.5). In addition, polystyrene surfaces can be derivatized with certain chemistries that will allow peptides and other nonprotein molecules to adhere to the surface to perform affinity assays in the wells of the plates.

We offer precoated plates to allow researchers an easy-to-use, consistent method for affinity purification or identification of specific molecules of interest. The plates offered include those specific for fusion proteins (6xHis, GST and GFP), antibodies (Protein A, Protein G, Protein A/G, Protein L, goat anti-mouse and goat anti-rabbit IgG), biotin (streptavidin and Thermo Scientific NeutrAvidin Protein) and those with reactive chemistries (maleic anhydride and maleimide) to allow binding of nonprotein samples that do not adsorb to the plastic microplate well surface. Only selected microplate products are featured in this handbook. For a complete selection of precoated plates, visit www.thermoscientific.com/pierce.

There are a variety of activated supports that allow a researcher to purify proteins and other biological molecules of interest either alone or when present in complexes with their binding partners. Many of these supports are discussed on the following pages.

Protein Purification Accessories

Protein Extraction, Binding and Elution Buffers

Protein purification is preceeded by expression of the target protein in a host organism. In *in vitro* protein expression, the simple introduction of DNA and a bacteriophage RNA polymerase to a cell free lysate initiates protein production, with no extraction reagents required before purification. For *in vivo* protein expression, such as in *E. coli* or tissue culture cells, expression can be driven from constituitive promoters or it can be induced chemically during bacterial growth or induced genetically through transient DNA transfection into tissue culture cells. For *in vivo* protein expression, the total protein content of a cell culture or tissue sample must be extracted from the cell's membranes and organelles before a chromatography resin can be used for purification. A list of Thermo Scientific Pierce Protein Extraction Reagents for different cell and tissue type is listed on page 7. Additionally, protease and phosphatase inhibitor cocktails that can be used to preserve protein integrity during extraction (see page 8).

Most affinity purification procedures involving protein:ligand interactions use binding buffers, such as phosphate buffered saline (PBS), at physiologic pH and ionic strength. This is especially true when antibody:antigen or native protein:protein interactions are the basis for the affinity purification. Once the binding interaction occurs, the support is washed with additional buffer to remove unbound components of the sample.

Nonspecific (e.g., simple ionic) binding interactions can be minimized by moderate adjustments to salt concentration or by adding low levels of detergent in the binding and/or wash buffer. Finally, elution buffer is added to break the binding interaction and release the target molecule, which is then collected in its purified form. Elution buffer can dissociate binding partners by extremes of pH (low or high), high salt (ionic strength), the use of detergents or chaotropic agents that denature one or both of the molecules, removal of a binding factor, or competition with a counter ligand. In most cases, subsequent dialysis or desalting is required to exchange the purified protein from elution buffer into a more suitable buffer for storage or downstream analysis. For more information on dialysis or desalting, download or request our a free high-performance dialysis technical handbook.

The most widely used elution buffer for affinity purification of proteins is 0.1M glycine•HCl, pH 2.5–3.0. This buffer effectively dissociates most protein:protein and antibody:antigen binding interactions without permanently affecting protein structure. However, some antibodies and proteins are damaged by low pH, so eluted protein fraction(s) should be neutralized immediately by collecting the fractions in tubes containing 1/10th volume of alkaline buffer such as 1M Tris•HCl, pH 8.5. Other elution buffers for affinity purification of proteins are listed in the accompanying table. In addition, we offer several preformulated binding and elution buffers designed for affinity purification involving antibodies.

Common elution systems for protein affinity purification.

Condition	Buffer
pH	100mm glycine•HCl, pH 2.5–3.0 100mm citric acid, pH 3.0 50–100mm triethylamine or triethanolamine, pH 11.5 150mm ammonium hydroxide, pH 10.5
Ionic strength and/or chaotropic effects	3.5–4.0M magnesium chloride, pH 7.0 in 10mm Tris 5M lithium chloride in 10mm phosphate buffer, pH 7.2 2.5M sodium iodide, pH 7.5 0.2–3.0 sodium thiocyanate
Denaturing	2–6M guanidine•HCl 2–8M urea 1% deoxycholate 1 % SDS
Organic	10% dioxane 50% ethylene glycol, pH 8–11.5 (also chaotropic)
Competitor	>0.1M counter ligand or analog

Cell Lysis Technical Handbook

This 50-page handbook provides protocols and technical and product information to help maximize results for protein/gene expression studies. The handbook provides helpful hints and troubleshooting for cell lysis, protein purification, cell fractionation, protease inhibitors and protein refolding. (# 1601756)

Thermo Scientific Pierce Protein Extraction Reagents.

Name	Description	Organisms/Samples
B-PER® - Bacterial Protein Extraction Reagent **Product # 90084**	Efficient, gentle lysis and extraction of soluble proteins from *E. coli* and other bacterial cells. Uses mild nonionic detergents to disrupt cells and solubilizing proteins without denaturation, eliminating the need for harsh mechanical procedures like sonication.	Gram(-) bacteria, *S. aureus, H. pylori, E. coli* strains BL21(D3)>JM109>DH5α>M15, Archaebacteria, nematodes and *Acinetobacter* sp.
B-PER II **Product # 78260**	Similar to B-PER, but optimized for low cell density, or for proteins with low expression levels.	Gram(-) bacteria, *S. aureus, H. pylori, E. coli* strains BL21(D3)>JM109>DH5α>M15, Archaebacteria, nematodes and *Acinetobacter* sp.
B-PER PBS **Product # 78266**	Similar to B-PER, but in Phosphate Buffer. This amine free formulation is ideal for amine-reactive labeling and/or crosslinking applications.	Gram(-) bacteria, *S. aureus, H. pylori, E. coli* strains BL21(D3)>JM109>DH5α>M15, Archaebacteria, nematodes and *Acinetobacter* sp.
B-PER with Enzymes **Product # 90078**	Similar to B-PER, but kit contains DNAse I and lysozyme, which improve cell membrane and DNA digestion for increased yields, and increases the recovery of large molecular weight proteins and insoluble proteins from inclusion bodies.	Gram(-) bacteria, *S. aureus, H. pylori, E. coli* strains BL21(D3)>JM109>DH5α>M15, Archaebacteria, nematodes and *Acinetobacter* sp.
B-PER Direct with Enzymes **Product # 90080**	Similar to B-PER with Enzymes, but bacteria can be lysed directly in cell culture media; ideal for screening 96-well microplate samples.	Gram(-) bacteria, *S. aureus, H. pylori, E. coli* strains BL21(D3)>JM109>DH5α>M15, Archaebacteria, nematodes and *Acinetobacter* sp.
Y-PER® - Yeast Protein Extraction Reagent **Product # 78990**	Easy-to-use solution gently disrupts the tough yeast cell wall in less than 20 minutes at room temperature, using a mild detergent. No mechanical disruption needed; yields more than twice as much protein as glass bead methods.	*S. cerevisiae, Schizo-saccharomyces pombe, C. albicans, B. subtilis, E. coli, P. pastoris, Strep. avidinii* and *Acinetobacter* sp.
Y-PER Plus **Product # 78998**	More stringent than Y-PER, but entire formulation (including detergent) are dialyzable.	Yeast (*S. cerevisiae*) and *Acinetobacter* sp.
M-PER® - Mammalian Protein Extraction Reagent **Product # 78501**	Highly efficient total protein extraction from cultured mammalian cells; extracts proteins in nondenatured state, enabling protein to be directly immunoprecipitated; amine-free and fully dialyzable; adhered cells can be directly lysed in plate or after scraping and washing in suspension.	Cultured mammalian cells, COS-7, NIH 3T3, Hepa 1-6, 293, CHO, MDA, MB231 and FM2
P-PER - Plant Protein Extraction Reagent **Product # 89803**	Contains organic lysing reagent and two aqueous reagents, which, in conjuction with mild mechanical agitation, effectively extract high quality protein extracts from plant leaves, stem, root, seed and flower cells without liquid nitrogen or harsh mechanical aids, such as mortar and pestle.	Multiple plant organs (leaf, stem, root, seed and flowers); multiple plant species (*Arabidopsis*, tobacco, maize, soybeans, peas, spinach, rice and other plant tissues); and fresh, frozen and dehydrated plant tissues
T-PER® - Tissue Protein Extraction Reagent **Product # 78510**	Simple, easy to use reagent for extracting total protein from tissue in 1:20 (w/v) of tissue to T-PER, using centrifugation to pellet cell/tissue debris. Mild detergent is dialyzable.	Heart, liver, kidney and brain.
I-PER - Insect Cell Protein Extraction Reagent **Product # 89802**	Optimized mild nonioinic detergent formulation provides maximum extraction of soluble proteins from insect cells; better yield than sonication; can be used for both suspended or adherent insect cells	Baculovirus-infected insect cells grown in suspension or monolayer culture.
NE-PER® - Nuclear and Cystoplasmic Extraction Kit **Product # 78833**	Obtain functional concentrated nuclear extracts and cytoplasmic fractions from mammalian cells and tissues in less than two hours, eliminating the need for freeze/thaw cycles, Dounce homogenization, lengthy centrifugation times and cold-room work.	Tissue: calf liver. Tissue: mouse heart, kidney, lung and liver; Cultured cells: epithelial (HeLa), fibroid (COS-7), kidney (NIH 3T3), liver (Hepa 1) and brain (C6).
Mem-PER® - Eukaryotic Membrane Protein Extraction Kit **Product # 89826**	Efficient, gentle reagents that solubilize and isolate membrane proteins from mammalian and yeast cells, as well as soft and hard tissues, in less than an hour. Minimal cross contamination (less than 10%) of hydrophilic proteins into the hydrophobic (membrane protein) fraction	Cultured cells: brain (C6), epithelial (HeLa), fibroblasts (NIH 3T3) and yeast (*S. cerevisiae*).
Subcellular Protein Fractionation Kit **Product # 78840**	Includes a combination of reagents for stepwise lysis of mammalian cells into functional cytoplasmic, membrane, soluble nuclear, chromatin-bound, and cytoskeletal protein fractions in a single kit; includes a stabilized nuclease and protease inhibitors. Extracts from each compartment have less than 15% contamination between fractions, with sufficient purity for studying protein localization and redistribution.	Cultured mammalian cells.
Mitochondrial Isolation Kit for Cultured Cells **Product # 89874**	Isolate intact mitochondria from cultured mammalian cells in approximately 40 minutes, with an optional Dounce homogenization protocol for increased yield.	Mammalian cells.
Mitochondrial Isolation Kit for Tissues **Product # 89801**	Isolate intact mitochondria from soft or hard tissue in less than 60 minutes, with an optional Dounce homogenization protocol for increased yield.	Heart, liver, kidney and brain.
Lysosome Enrichment Kit for Tissues and Cells **Product # 89839**	Uses density gradient centrifugation to separate lysozyme from contaminating celluar structures in both mammalian cells and soft and hard tissue.	Tissues and cultured cells.
Peroxisome Enrichment Kit for Tissues **Product # 89840**	Uses density gradient centrifugation to separate peroxisome from contaminating celluar structures in both soft and hard tissue.	Heart, liver, kidney and brain.
Nuclei Enrichment Kit for Tissue **Product # 89841**	Uses density gradient centrifugation to separate nuclei from contaminating celluar structures in both soft and hard tissue	Heart, liver, kidney and brain.
Pierce® RIPA Buffer **Product # 89900**	Extracts cystoplasmic, membrane, and nuclear proteins from cultured mammalian cells; can be used for both plated cells and cells pelleted from suspension cultures. Protease and phosphatase inhibitors are compatible with this formulation.	Cultured mammalian cells and cytoplasmic, membrane and nuclear proteins.
Pierce IP Lysis Buffer **Product # 87787**	Gently extracts cytoplasmic, membrane and nuclear proteins while maintaining protein complexes for immunoprecipitation (IP), Pulldowns, and co-IP; does not liberate DNA which can cause high viscosity.	Cultured mammalian cells.

For more details on these products, request the Cell Lysis Technical Handbook (#1601756) at www.thermoscientific.com/pierce

Protease and Phosphatase Inhibitors for Protein Purification

Thermo Scientific Halt Protease and Phosphase Inhibitors

Our broad-spectrum protease and phosphatase inhibitor cocktails and individual protease inhibitors accommodate specific and general needs in cell lysis and protein extraction methods.

Highlights

- Halt Protease Inhibitor Cocktails target serine, cysteine and aspartic acid proteases and aminopeptidases. Metalloproteases are inhibited by the optional addition of EDTA. Individual protease inhibitors targeting separate classes of proteases are also available for custom cocktail development.
- Halt™ Phosphatase Inhibitor Cocktails contain chemical compounds that target serine/threonine and tyrosine phosphatases.
- The Halt Protease and Phosphatase Inhibitor Cocktail prevents protein degradation and preserves phosphorylation simultaneously, providing protection that is similar to the individual cocktails.

For a complete listing of the Halt Protease and Phosphatase Inhibitors at www.thermoscientific.com.

Buffers for Protein Purification

Thermo Scientific BupH Pack Dry Blend Buffers

BupH® Packs are pre-blended and pre-measured dry mixtures of commonly needed buffers that are easy to prepare; simply empty contents of foil envelope pack into a beaker, add ultrapure water, and stir to dissolve. The packs eliminate weighing time and tedious pH adjustments. BupH Pack Dry Blend Buffers are offered for use in common laboratory techniques and to support other Pierce Protein Research Products.

Highlights:

- **Convenient** – dissolve contents of one envelope in 500ml of water and the buffer is ready to use
- **Save time and trouble** – no weighing, no pH adjustment, no need to stock individual components and no need to make and store large volumes of stock solution in advance of daily needs
- **Long shelf life** – stocking and storage as dry packs eliminates concerns about long-term stability of stock solutions
- **Eliminate variables** – our quality control ensures that every pack will yield the same, consistent buffer

Ordering Information

Product #	Description	Pkg. Size	Applications	Formulation of each pack after reconstitution
28384	Borate Buffer	40 packs	Protein modification procedures that require amine-free buffer at alkaline pH	500mL of 50mM borate, pH 8.5
28382	Carbonate-Bicarbonate Buffer	40 packs	Microplate protein and antibody coating for ELISA or RIA	500mL of 0.2M carbonate-bicarbonate, pH 9.4
28388	Citrate-Carbonate Buffer	10 packs	Protein immobilization to UltraLink® Biosupport (Product # 53110)	100mL of 0.6M sodium citrate, 0.1M sodium carbonate, pH 9
28386	Citrate-MOPS Buffer	10 packs	Protein immobilization to UltraLink Biosupport (Product # 53110)	100mL of 0.6M sodium citrate, 0.1M MOPS, pH 7.5
28390	MES Buffered Saline	10 packs	Crosslinking using carbodiimide (EDC, Product # 22980)	500mL of 0.1M MES, 0.9% NaCl, pH 4.7
28372	Phosphate Buffered Saline (PBS)	40 packs	Crosslinking and biotinylation requiring amine-free buffer	500mL of 0.1M sodium phosphate, 0.15M NaCl, pH 7.2
28374	Modified Dulbecco's PBS	40 packs	Wash buffers and antibody diluents for ELISA, Western blotting and other immunoassays	500mL of 8mM sodium phosphate, 2mM potassium phosphate, 0.14M NaCl, 10mM potassium chloride, pH 7.4
28379 28376	Tris Buffered Saline	10 packs 40 packs	Wash buffers and antibody diluents for ELISA, Western blotting and other immunoassays	500mL of 25mM Tris, 0.15M NaCl, pH 7.2
28378	Tris-Glycine-SDS Buffer	40 packs	Running buffer for Tris-Glycine gel electrophoresis	500mL of 25mM Tris, 192mM glycine, 0.1% SDS, pH 8.3
28398	Tris-HEPES-SDS Running Buffer 10	10 packs	Running buffer for Tris-HEPES electrophoresis with Precise™ Precast Gels	500mL of 100mM Tris, 100mM HEPES, 3mM SDS, pH 8±0.25
28380	Tris-Glycine Transfer Buffer	40 packs	Running buffer for gel to membrane electrophoretic transfer	500mL of 25mM Tris, 192mM glycine, pH 8 (use 20% methanol to dissolve)

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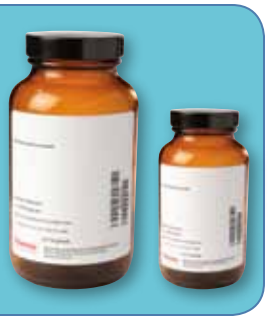
Call our Bulk and Custom department at 1-800-874-3723 or +1 815-968-0747 ext. 300 for pricing and information. Or, visit www.thermoscientific.com/protein-custom



Thermo Scientific Concentrated Liquid Buffers

Save counter space and time.

Pierce 10X and 20X Concentrated Buffers are ready to use without having to reconstitute with ultrapure water and, in the case of Tris-Glycine Buffer, methanol. Buffers are designed for use in dialysis, cross-linking, enzyme assays, ELISAs, immunohistochemistry, protein plate-coating, biotinylation and other applications. Keep our concentrated buffers close at hand to cover your lab's research needs.

Highlights:

- **Easy to use** – no packets to open and no powder to dissolve
- **Increased accuracy** – eliminates the possibility of powder remaining in a packet
- **Saves time** – 10X or 20X concentration eliminates time spent waiting for powder to dissolve
- **Saves space** – storage as concentrated stock minimizes bench space needed for large volume solutions

PBS

Tris-Glycine-SDS

Tris-Hepes-SDS

Ordering Information

Product #	Description	Pkg. Size	Applications	1X Formulation
28341	20X Borate Buffer	500mL	Ideal for protein modification procedures requiring amine free buffer at alkaline PH	500mL of 50mM borate, pH 8.5
28344	20X Modified Dulbecco's PBS Buffer	500mL	Used for wash buffers and antibody diluents in applications such as ELISA, Western blotting and other immunoassays	8mM Sodium Phosphate, 2mM Potassium Phosphate, 0.14M NaCl, 100mM KCl, pH 7.4
28346	20X Modified Dulbecco's PBS Tween-20 Buffer	500mL	A wash buffer for ELISA, Western and other Immunoassays as well as a blocking buffer for plate based assays	8mM Sodium Phosphate, 2mM Potassium Phosphate, 0.14M NaCl, 100mM KCl, 0.05% Tween-20, pH 7.4
28348	20X Phosphate Buffered Saline	500mL	Its ionic strength makes it ideal for crosslinking and biotinylation requiring amine free buffer	0.01M Sodium Phosphate, 0.15M NaCl, pH 7.5
28352	20X PBS Tween-20 Buffer	500mL	A wash buffer for ELISA, Western and other Immunoassays as well as a blocking buffer for plate based assays	0.01M Sodium Phosphate, 0.15M NaCl, 0.05% Tween-20, pH 7.5
28354	20X TAE Buffer	500mL	Historically the most common buffer used for agarose gel electrophoresis in the analyses of nucleic acids	0.04M Tris, 0.04M Acetate, 0.001M EDTA, pH 8.2-8.4
28355	10X TBE Buffer	1L	Often used for agarose gel electrophoresis in the analysis of nucleic acids	0.089M Tris, 0.089 M Borate, 0.002M EDTA, pH 8.2-8.4
28358	20X TBS Buffer	500mL	Used for wash buffers and antibody diluents in applications such as ELISA, Western blotting and other immunoassays	25mM Tris, 0.15M NaCl, pH 7.2
28360	20X TBS Tween-20 Buffer	500mL	A wash buffer for ELISA, Western and other Immunoassays as well as a blocking buffer for plate based assays	25mM Tris, 0.15M NaCl, 0.05% Tween-20, pH 7.5
28362	20X Tris/HEPES/SDS Buffer	1L	A running buffer for Tris-HEPES electrophoresis with Precise Precast Gels	0.025 M Tris, 0.192 M Glycine, 0.1% SDS, pH 8.5
28363	10X Tris-Glycine Buffer	1L	Great for electrophoretic transfer from gel to membrane	0.025M Tris, 0.192M Glycine, pH 8.5
28368	20X Tris/HEPES/SDS Buffer	500mL	A running buffer for Tris-HEPES electrophoresis with Precise Precast Gels	0.1M Tris, 0.1M HEPES, 3mM SDS, pH 8+0.25

Protein Purification Accessories

Spin Cups and Columns

Thermo Scientific Pierce Spin Columns are convenient tools for manipulating small volumes of affinity supports (5–500µL) for protein purification. Add the affinity resin and sample to one of the columns, use a microcentrifuge to efficiently wash away contaminants and elute your purified sample without losing any resin in the process. Spin columns allow you to affinity-purify more protein in less time!

Highlights:

- Efficient washing of samples means fewer washes are needed to remove contaminating proteins
- Efficient elution of samples means more antigen and co-precipitated proteins are recovered
- No resin loss means more consistent IP and co-IP results
- No need to decant supernatant from IP or co-IP pellet
- Spin protocols drastically reduce the time required for IPs and co-IPs
- Low protein-binding polypropylene column construction minimizes nonspecific binding

Spin Cups – Paper Filter

Paper filter with collection tubes.

Highlights:

- Paper filters are resistant to clogging from cellular debris
- Column Volume: 600µL
- Resin Volume: 20–300µL
- Filter Type: Paper, ~10µm pore size
- Cap Type: Collection tube cap fits onto inserted spin cup

Spin Cups – Cellulose Acetate Filter

Cellulose acetate filter with collection tubes.

Highlights:

- Used in our IP and Co-IP Kits
- Column Volume: 800µL
- Resin Volume: 20–400µL
- Filter Type: Cellulose acetate, 0.45µm pore size
- Cap Type: Collection tube cap fits onto inserted spin cup

Spin Columns – Screw Cap

Screw cap with Luer-Lok® Adaptors.

Highlights:

- Luer-Lok Adaptors allow these columns to be used for syringe-based purifications
- Column Volume: 900µL
- Resin Volume: 20–400µL
- Filter Type: Polyethylene, ~10µm pore size
- Small & large frit options for different sample sizes
- Cap Type: O-ring screw top caps; press-in bottom plugs

Spin Columns – Snap Cap

Snap cap with collection tubes.

Highlights:

- Used in the Cell Surface Protein Isolation and Glycoprotein Isolation Kits
- Column Volume: 1,000µL
- Resin Volume: 20–500µL
- Filter Type: Polyethylene, ~30µm pore size
- Cap Type: Snap cap on column (no cap on collection tube); press-on bottom caps

Micro-Spin Columns

Highlights:

- Column Volume: 400µL
- Resin Volume: 5–100µL
- Filter Type: Polyethylene, ~30µm pore size
- Cap Type: O-ring screw top caps; press-on bottom caps

Ordering Information

Product #	Description	Pkg. Size
69700	**Spin Cups – Paper Filter** Includes cups and collection tubes	50/pkg
69715	**Microcentrifuge Tubes** Collection Tubes for Product # 69700	72/pkg
69702	**Spin Cups – Cellulose Acetate Filter** Includes cups and collection tubes	50/pkg
69720	**Microcentrifuge Tubes** Collection Tubes for Product # 69702	72/pkg
69705	**Spin Columns – Screw Cap with Luer-Lok Adaptors** Includes: Spin Columns, Screw Caps and Column Plugs Luer-Lok Adaptors Large Frits (6.8mm diameter 10µm pore size) Small Frits (2.7mm diameter 10µm pore size) Large and Small Frit Tools	Kit 25 each 5 each 25 each 25 each 1 each
69725	**Spin Columns – Snap Cap with Collection Tubes** Includes: Spin Columns and Bottom Caps Collection Tubes	Kit 50 each 100 each
89879	**Micro-Spin Columns**	50/pkg

Disposable Plastic Columns

Automatic "stop-flow" action provided by porous polyethylene discs prevents column beds from drying out.

Highlights:

- Supplied complete with porous polyethylene discs, stoppers, end caps
- Compatible with most types of aqueous buffer eluents commonly used in chromatography
- Can be pre-packed and stored until needed

Ordering Information

Product #	Description	Pkg. Size
29920	**Disposable Polystyrene Columns** Ideal for packing 0.5–2.0mL bed volumes.	100/pkg
29922	**Disposable Polypropylene Columns** Ideal for packing 1–5mL bed volumes.	100/pkg
29924	**Disposable Polypropylene Columns** Ideal for packing 2–10mL bed volumes.	100/pkg
29923	**Disposable Polypropylene Funnels** Buffer reservoirs that fit Product #'s 29920, 29922 and 29924.	50/pkg
29925	**Disposable Column Trial Pack** Includes accessories plus two each of Product #'s 29920, 29922 and 29924 and one of Product # 29923.	Trial Pack

Centrifuge Columns

Efficiently handle a wide variety of resin volumes for affinity purification! Thermo Scientific Pierce Centrifuge Columns are convenient tools for handling 40µL–10mL of an affinity purification support. Add the affinity resin to one of the polypropylene columns, remove the twist-off bottom and allow the resin to pack itself. Then add your sample and allow it to bind to the support. Use a centrifuge to efficiently wash away any contaminants and elute your purified protein.

Pierce Centrifuge Columns allow you to use a spin-column format in addition to traditional gravity flow to reduce the time required for column washing and elution. This accelerates sample processing time and makes multiple-sample processing possible. Centrifuge columns allow you to affinity-purify more protein in less time!

Centrifuge columns are made from low protein-binding polypropylene for compatibility with protein purification, and they fit into standard centrifuge tubes for use in any centrifuge.

Applications for Centrifuge Columns:

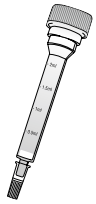
- Affinity purification/affinity chromatography
- Immunodepletion
- Spin desalting

0.8mL Centrifuge Columns

Highlights:

- Total Volume: 800µL
- Resin Volume: 40–400µL
- Filter Type: Polyethylene, ~30µm pore size
- Receiver Tube: Fits standard microcentrifuge tubes (e.g., Product # 69720)
- Cap Type: O-ring screw-top cap
- Twist-off bottom

2mL Centrifuge Columns



Highlights:

- Total Volume: 5mL (2mL resin bed, 3mL reservoir)
- Resin Volume: 2mL
- Filter Type: Polyethylene, ~30µm pore size
- Receiver Tube: Fits standard 15mL conical centrifuge tubes
- Cap Type: Screw-top cap
- Twist-off bottom and press-on cap to reseal

5mL Centrifuge Columns

Highlights:

- Total Volume: 8mL (5mL resin bed, 3mL reservoir)
- Resin Volume: 5mL
- Filter Type: Polyethylene, ~30µm pore size
- Receiver Tube: Fits standard 15mL conical centrifuge tubes
- Cap Type: Screw-top cap
- Twist-off bottom and press-on cap to reseal

10mL Centrifuge Columns

Highlights:

- Total Volume: 22mL (10mL resin bed, 12mL reservoir)
- Resin Volume: 10mL
- Filter Type: Polyethylene, ~30µm pore size
- Receiver Tube: Fits standard 50mL conical centrifuge tubes
- Cap Type: Screw-top cap
- Twist-off bottom and press-on cap to reseal

Ordering Information

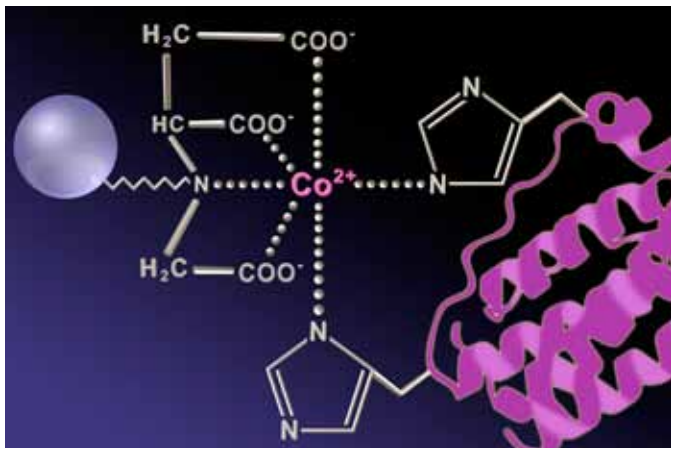
Product #	Description	Pkg. Size
89868	**Centrifuge Columns, 0.8mL capacity** Includes: Pierce Centrifuge Columns Screw Caps	Kit 50 each 50 each
89869	**Centrifuge Columns, 0.8mL capacity** Includes: Pierce Centrifuge Columns Screw Caps	Kit 4 x 50 each 4 x 50 each
89896	**Centrifuge Columns, 2mL capacity** Includes: Pierce Centrifuge Columns Screw Caps and Tips	Kit 25 each 25 each
89897	**Centrifuge Columns, 5mL capacity** Includes: Pierce Centrifuge Columns Screw Caps and Tips	Kit 25 each 25 each
89898	**Centrifuge Columns, 10mL capacity** Includes: Pierce Centrifuge Columns Screw Caps and Tips	Kit 25 each 25 each
69707	**Column Extender** Fits 89896, 89897 and 89898. Increases column capacity by 35mL	10 each

Fusion Protein Purification

Fusion Protein Purification

Cultures of *E. coli* or *Picchia* are common vehicles for protein expression. They are low cost and low maintenance platforms which can be easily scaled up to deliver protein at milligram to gram yields. These microoganisms are also very easy to transform with a DNA vector containing the gene of interest. Typically, researchers use common expression vectors which possess the proper promoter elements for expression and inclusion of an affinity tag sequence. The affinity tag sequence is cloned in frame with the DNA sequence of the target protein, and will flank either the N- or C-terminus. The two most common affinity tags are polyhistidine (6xHis) and glutathione-S-trasferase (GST).

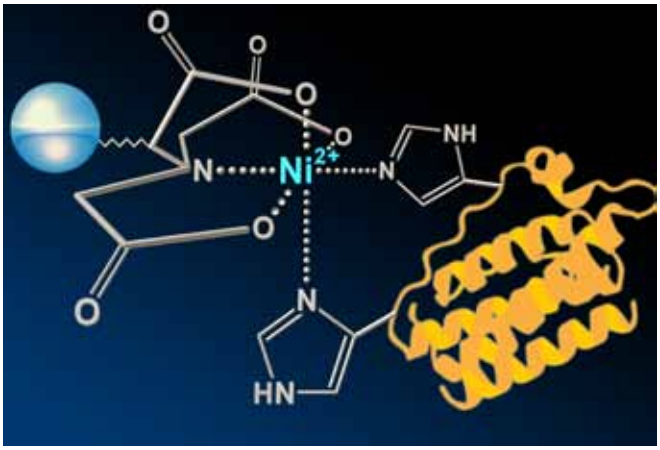
Polyhistidine Purification



Cobalt-histidine binding.

The polyhistidine tag is a sequence of five to nine histidine amino acids attached to the terminus of a target protein. The polyhistidine tag is purified using immobilized metal affinity chromatography (IMAC). For histidine tag purification, either nickel or cobalt is immobilized onto a solid chromatography resin. While the two metals can be used interchangeably, typically nickel has a higher binding capacity whereas cobalt binds less non-specific protein to deliver a purer final protein.

IMAC resins work by charge interactions with the nitrogen atoms on the histidine amino acid side chain to bind and immobilize the histidine-tagged protein from a cell lysate. The incorporation of multiple histidine residues as an affinity tag is designed to improve this charge association. Because IMAC affinity for histidine residues is not dependent on the secondary structure of the protein, IMAC purification can be performed under denaturing conditions. IMAC purification is, however, sensitive to pH and the presence of chelators and reducing agents. See Table 1 for a list of common interfering agents and their concentration tolerance for both nickel and cobalt resin.



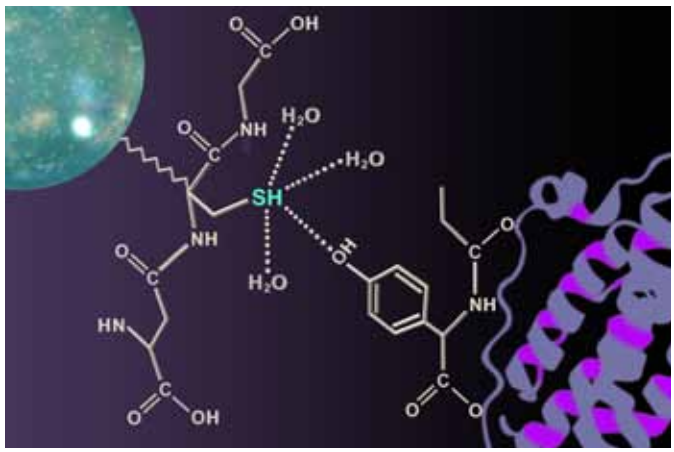
Nickel-histidine binding.

Table 1. Common interfering agents for Ni-NTA and cobalt resins.

Reagent	Tolerance for Ni-NTA resin	Tolerance for Cobalt resin
ß-Mercaptoethanol	10mM	10mM
CHAPS	1%	1%
Ethanol	30%	30%
Ethylene glycol	30%	30%
HEPES	50mM	50mM
Glycerol	20%	20%
Guanidinium	6M	6M
Imididazole	500mM	200mM at pH 7.0-8.0 for elution
KCl	500mM	500mM
MES	20mM	20mM
MOPS	50mM	50mM
NaCl	1.0M	1.0M
NP-40	1%	1%
SDS	1%	1%
TRIS	50mM	50mM
Triton®-X-100	<1%	<1%
Urea	8M	8M

Once immobilized, imidazole is used to disrupt the charge attractions between the immobilized metal affinity chromatography resin and the histidine-tagged protein. The eluted histidine-tagged protein can be easily cleaned-up using a desalting column or dialysis cassette to remove imidazole. Often trace amounts of imidazole are included in the protein binding and wash steps to compete away binding of endogenous proteins with multiple histidines.

Glutathione-S-transferase Purification



GST binding.

GST is a 26kDa endogenous enzyme found in both prokaryotes and eukaryotes. In the cell GST detoxifies reactive oxygen species such as free radicals and peroxides. GST performs this task by neutralizing reactive oxygen species with the antioxidant glutathione (GSH). GSH is an endogenous tripeptide (Glu-Cys-Gly) containing a cysteine residue, whose sulfhydryl side chain functions as a reducing agent.

GST makes an ideal affinity tag because of its strong binding to reduced glutathione. GST possess numerous tyrosine residues in the in the GSH binding pocket, and one of these tyrosines hydrogen bonds to the substrate glutathione forming a stable complex. *In vivo*, GST would transfer glutathione to a reactive oxygen species and neutralize it.

As a research tool, scientists have taken advantage of the strong interaction between GST and GSH to selectively extract recombinant proteins. In this strategy, glutathione is immobilized onto a solid support (typically an agarose bead) and the amino acid sequence for the enzyme GST is cloned into either the C- or N-terminus of the target gene (commonly using the pGEX®-series of expression vectors). After binding of the GST-tagged fusion protein to the immobilized glutathione agarose, excess reduced glutathione is introduced (typically between 10-50mM) to compete off the target protein. Again, simple dialysis or desalting columns can be used to clean up the final GST-tagged protein. For reasons that have not been fully characterized in the literature, the structure of the GST fusion tag often degrades upon denaturation and reduction for protein gel electrophoresis (e.g., SDS-PAGE). As a result, electrophoresed samples often appear as a ladder of lower MW bands below the full-sized fusion protein.

In contrast to polyhistidine purification, GST purification requires the target protein maintain native tertiary structure. Additionally, the GST tag is quite large (26kDa) compared to the six histidines which comprise a typical polyhistidine tag. To circumvent the problems associated with a 26kDa appendage, selective proteases are used to cleave the GST tag from the GST-fusion protein. These proteases include Factor Xa (Product # 32520) or thrombin.

His-Tagged Protein Purification Resin

A cost-effective Ni-NTA resin is now available.

The expression and purification of recombinant proteins is central to protein regulation, structure and function studies. The majority of recombinant proteins are expressed as fusions with short affinity tags with the most popular being the polyhistidine (6xHis) tag. The method used to purify recombinant His-tagged proteins is immobilized metal affinity chromatography (IMAC), consisting of chelating resins charged with either nickel or cobalt ions that coordinate with the histidine side chains. The new Thermo Scientific HisPur Ni-NTA Resin complements the HisPur™ Cobalt Resin. Ni-NTA resins are the most common IMAC resin choice for 6xHis-tagged protein purifications because of the four metal-binding sites on the chelate, which enables high-protein binding and low-metal ion leaching.

Highlights:

- **High capacity** – bind up to 60mg of 6xHis-tagged protein per milliliter of resin
- **Versatile** – purify proteins using native or denaturing conditions
- **Compatible** – use with Thermo Scientific Cell Lysis Reagents and a variety of buffer additives
- **Flexible** – available in multiple formats, including bulk resin, spin columns and chromatography cartridges
- **Cost-effective** – reuse the same batch of resin at least five times
- **Easy to use** – pre-formulated buffers available for kit formats

His-tagged green fluorescent protein (GFP) was recovered in similar or greater purity and yield compared to other commercially available nickel-chelated resins (Figure 1). HisPur Ni-NTA Resin is also cost-effective because it can be used at least five times without loss in performance (Figure 2). The resin is amenable to chromatography-cartridge format, resulting in a sharp elution peak evident in the chromatogram (Figure 3). When HisPur Ni-NTA Resin is compared to HisPur Cobalt Resin (Product # 90090) and Ni-IDA resin using the batch-bind method, the results depend on protein expression level (Table 2 and Figure 4). When purifying a low-expression protein, such as 6xHis-AIF2, there is a dramatic difference in purity. HisPur Cobalt Resin yields the purest protein followed by HisPur Ni-NTA Resin with Ni-IDA being the least pure. When purifying 6xHis-GFP, a high expresser, there was minimal difference between HisPur Ni-NTA and Cobalt Resins. Similar results were obtained using spin purification (data not shown).

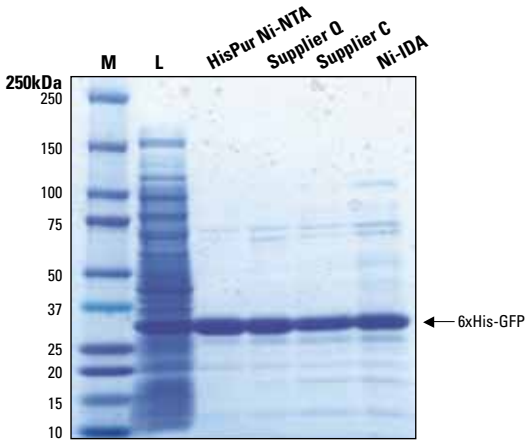


Figure 1. Thermo Scientific HisPur Ni-NTA resin is comparable to or performs better than other suppliers' nickel resins. Bacterial lysate (12mg total protein) containing over-expressed 6xHis-green fluorescent protein (GFP) was applied to HisPur Ni-NTA Resin (200µL) and purified by the batch-bind method as described. The same amount of total protein was applied to Supplier Q, Supplier C and Ni-IDA resins per the manufacturers' instructions. Gel lanes were normalized to equivalent volume. **M =** molecular-weight marker and **L =** lysate load.

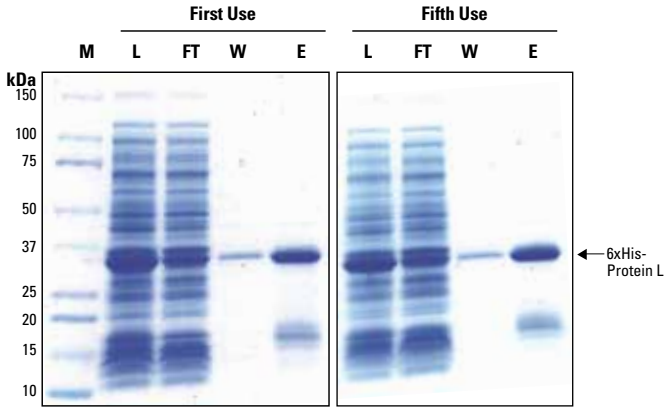


Figure 2. Thermo Scientific HisPur Ni-NTA Resin can be used at least five times without losing performance. Bacterial lysate (20mg total protein) containing over-expressed 6xHis-Protein L was applied to HisPur Ni-NTA Resin (200µL) and purified by the batch-bind method as described. Before each reuse, the resin was washed with 20mm MES buffer, 0.1 M NaCl; pH 5 (1mL) and followed by a water wash (1mL). Gels lanes were normalized to equivalent volume. **M =** molecular-weight marker, **L =** lysate load, **FT =** flow-through and **E =** elution.

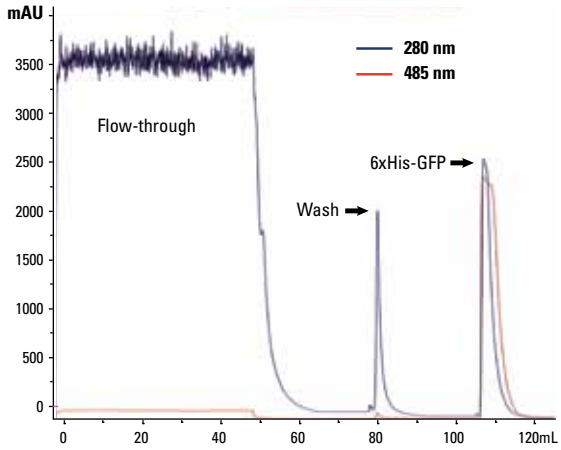


Figure 3. Thermo Scientific HisPur Ni-NTA Chromatography Cartridges provide an automatable solution for obtaining high-purity proteins. Bacterial lysate (140mg total protein) containing over-expressed 6xHis-GFP was diluted with equilibration buffer and applied to a HisPur Ni-NTA Chromatography Cartridge at a flow rate of 1mL/min. The cartridge was washed with PBS, 60mm imidazole until the baseline absorbance was reached. 6xHis-GFP was eluted with PBS, 300mm imidazole. Elution was monitored at 280nm (blue line) and 485nm (red line).

Table 2. Elution fractions were analyzed for protein content using the Thermo Scientific Coomassie Plus (Bradford) Protein Assay Kit (Product # 23236). Purity was determined by analyzing the stained SDS-PAGE gel (Figure 4) with densitometry software.

Sample	Resin	Total yield (mg)	Purity
6xHis-AIF2	HisPur Ni-NTA	0.5	32%
	Ni-IDA	0.5	25%
	HisPur Cobalt	0.4	49%
6xHis-GFP	HisPur Ni-NTA	0.8	90%
	Ni-IDA	0.6	52%
	HisPur Cobalt	0.7	91%

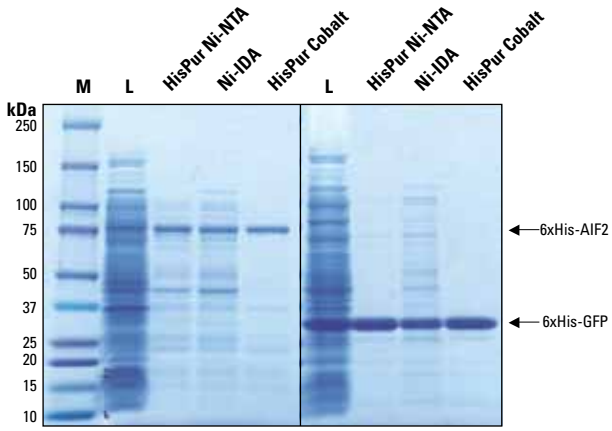


Figure 4. Thermo Scientific HisPur Cobalt Resin produces the most pure results. Bacterial lysate containing over-expressed 6xHis-AIF2 (6mg total protein) or 6xHis-GFP (4mg total protein) was applied to HisPur Ni-NTA Resin (200µL) and purified by the batch-bind method as described. The same amount of total protein was applied to Ni-IDA and HisPur Cobalt Resins and purified as described in the instructions. Gels lanes were normalized to equivalent volume. **M =** molecular-weight marker, **L =** lysate load.

The HisPur Ni-NTA Resin offers a cost-effective alternative to other commercially available nickel-IMAC resins, without compromising yield, purity or performance. The HisPur Ni-NTA and Cobalt Resins are available in multiple formats to accommodate a variety of applications and purification volumes.

Ordering Information

Product #	Description	Pkg. Size
88221	HisPur Ni-NTA Resin	10mL
88222	HisPur Ni-NTA Resin	100mL
88223	HisPur Ni-NTA Resin	500mL
88224	HisPur Ni-NTA Spin Columns, 0.2mL	25 columns
88225	HisPur Ni-NTA Spin Columns, 1mL	5 columns
88226	HisPur Ni-NTA Spin Columns, 3mL	5 columns
88227	HisPur Ni-NTA Purification Kit§, 0.2mL	25 columns
88228	HisPur Ni-NTA Purification Kit§, 1mL	5 columns
88229	HisPur Ni-NTA Purification Kit§, 3mL	5 columns
90098	HisPur Ni-NTA Chromatography Cartridges, 1mL	5 cartridges
90099	HisPur Ni-NTA Chromatography Cartridges, 5mL	2 cartridges

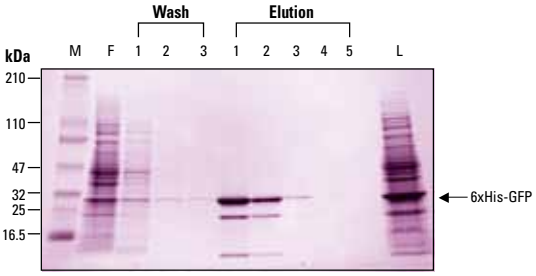
§ For complete ordering information and kit components, please visit www.thermoscientific.com/pierce

Cobalt Resin, Spin Columns and Chromatography Cartridges

Specific, fast and gentle purification of His-tagged proteins.

The preferred method for purifying recombinant His-tagged proteins is immobilized metal affinity chromatography (IMAC). Traditionally, chelating chromatography resins are charged with either nickel or cobalt ions that coordinate with the histidine side chains in the 6xHis-tag. HisPur Cobalt Resin is a tetradentate chelating resin charged with cobalt that binds His-tagged proteins with high specificity and releases them under lower imidazole concentrations than required with nickel resins (see Figure 5). HisPur Cobalt Resin can be used to obtain high-purity proteins with no metal contamination.

The HisPur Chromatography Cartridges are convenient, reliable and ready-to-use pre-packed devices for the isolation of proteins in solution and purification of His-tagged fusion proteins. The HisPur Cartridges are compatible with automated LC instrumentation, such as the ÄKTA® and BioLogic Systems, and adapt to manual syringe processing.



Thermo Scientific HisPur Cobalt Resin is specific for His-tagged proteins and allows mild, efficient elutions. Bacterial lysate (1.0mg total protein) was applied to a 200µL bed volume of HisPur Cobalt Resin in a spin column. Gel lanes were normalized to equivalent volume. **M =** Molecular Weight Markers (Product # 26691), **L =** lysate load and **F =** flow-through.

Highlights:

- **High purity** – obtain pure protein without optimizing imidazole washing conditions
- **Specificity** – cobalt:chelate binding core binds fewer contaminants, resulting in lower background than nickel (see Table 3)
- **Low metal leaching** – no metal contamination in eluted sample
- **Versatility** – purify proteins under native or denaturing conditions; compatible with cell lysis reagents and a variety of buffer additives
- **Flexibility** – available as bulk resin or predispensed columns compatible with both spin and gravity-flow formats
- **Cost effective** – reuse or discard
- **Superior** – performs better than other commercially available IMAC Resins (see Figure 5)

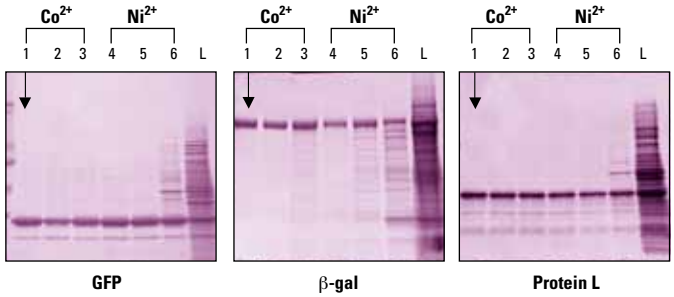
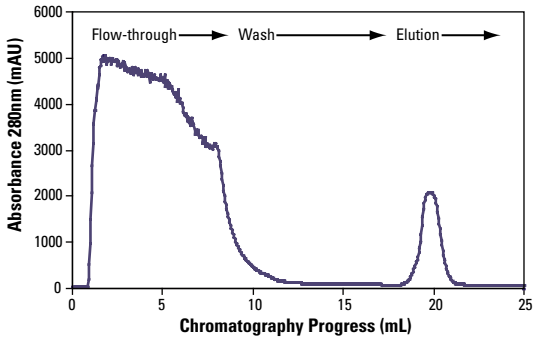


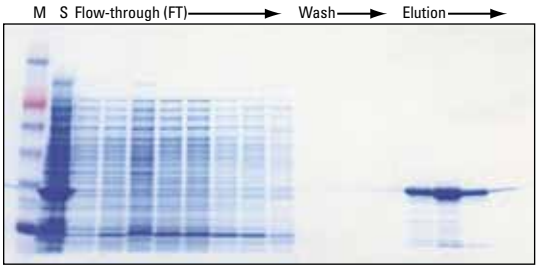
Figure 5. Thermo Scientific HisPur Cobalt Resin outperforms other IMAC resins. Comparable yield and higher purity obtained with HisPur Cobalt Resin (lane 1) compared to other IMAC resins (lanes 2 to 6). Cell lysates containing over-expressed recombinant 6xHis-tagged protein were prepared in B-PER Bacterial Protein Extraction Reagent (Product # 78243) and Protease Inhibitors (Product # 78410). Protein concentrations were determined by Coomassie Plus Protein Assay (Product # 23238). *E. coli* lysates containing over-expressed His-tagged GFP, β-galactosidase or Protein L were applied to 0.2mL bed volumes of each IMAC resin in spin column format. Binding, wash and elution buffers were prepared and used per each manufacturers' instructions. The first elution fraction for each IMAC resin was analyzed by SDS-PAGE and protein purity determined by densitometry. Gel lanes were normalized to equivalent volume. **Lanes: 1**= HisPur Cobalt Resin, **2**= supplier C cobalt resin, **3**= supplier S cobalt resin, **4**= supplier G nickel resin, **5**= supplier Q nickel resin, **6**= Ni-IDA and **L**= lysate load.

HisPur Cobalt Cartridge Performance Data:

Chromatography Profile



Electrophoresed Fractions



Purification of 6xHis-GFP from *E. coli* lysate using a Thermo Scientific HisPur Cobalt Cartridge. His-tagged green fluorescent protein (GFP) was extracted from *E. coli* using Thermo Scientific B-PER Bacterial Protein Extraction Reagent in Phosphate Buffer (Product # 78266) containing Halt Protease Inhibitor Cocktail, EDTA-Free (Product # 78415). The lysate was diluted 1:1 with equilibration/wash buffer (50mM sodium phosphate, 300mM sodium chloride, 10mM imidazole, pH 7.4) and applied to a Thermo Scientific HisPur Cobalt Chromatography Cartridge at a flow rate of 0.3mL/min. The cartridge was washed with equilibration/wash buffer until the baseline absorbance at A_{280} was reached. His-tagged GFP was eluted (50mM sodium phosphate, 300mM sodium chloride, 150mM imidazole; pH 7.4) and selected fractions were analyzed by SDS-PAGE and Thermo Scientific GelCode Blue Stain Reagent (Product # 24592). **M =** MW Marker; S = non-fractionated lysate; **FT =** flow-through.

Ordering Information

Product #	Description	Pkg. Size
89967	**HisPur Cobalt Spin Columns, 0.2mL**	25 columns
89968	**HisPur Cobalt Spin Columns, 1mL**	5 columns
89969	**HisPur Cobalt Spin Columns, 3mL**	5 columns
89964	**HisPur Cobalt Resin**	10mL bottle
89965	**HisPur Cobalt Resin**	100mL bottle
89966	**HisPur Cobalt Resin**	500mL bottle
90090	**HisPur Purification Kit, 0.2mL**	25 columns
90091	**HisPur Purification Kit, 1mL**	5 columns
90092	**HisPur Purification Kit, 3mL**	5 columns
90093	**HisPur Chromatography Cartridges**	5 x 1mL
90094	**HisPur Chromatography Cartridges**	2 x 5mL

Table 3. Thermo Scientific HisPur Cobalt Resin yields more His-tagged protein and higher purity than other Co^{2+} and Ni^{2+} IMAC Resins.

	His-GFP		His-β-Gal		His-Protein L	
	Yield (µg)*	Purity (%)**	Yield (µg)*	Purity (%)**	Yield (µg)*	Purity (%)**
Thermo Scientific HisPur Cobalt Resin	298	87	78	93	42	77
Supplier C Cobalt Resin	206	78	26	90	35	76
Supplier S Cobalt Resin	211	85	27	65	38	77
Supplier G Nickel Resin	239	84	42	83	29	68
Supplier Q Nickel Resin	242	85	24	48	30	72
Ni^{2+}-IDA Resin	70	37	6	16	17	46

** Recovered from a 5mg total protein load (total protein yield x purity).*

*** Purity of the first elution fraction.*

High-quality purification of GST-fusion proteins

Multiple formats available to meet your purification needs

Purification of glutathione S-transferase (GST) fusion proteins using glutathione agarose beads provides one-step, high-purity affinity purification. The Thermo Scientific Pierce Glutathione Agarose is composed of 6% crosslinked beaded agarose with glutathione (GSH) immobilized by its central sulfhydryl. GST-fusion proteins are purified with high yield because of the 12-atom GSH linker that
minimizes steric hindrance. The Pierce Glutathione Agarose is available in resin slurry packages, spin columns, complete purification kits and FPLC-ready chromatography cartridges.

Highlights:

- **High capacity** – binds at least 40mg of pure GST per milliliter of resin
- **Cost-effective** – resin is economically priced and can be reused at least five times without reduction in performance
- **High yield and purity** – each milliliter of resin can purify ≥ 10mg of GST-tagged protein from lysates with > 90% purity
- **Compatible** – validated and effective with Thermo Scientific Cell Lysis Reagents
- **Versatile** – purify lysates or pulldown protein-protein interactions
- **Flexible** – available in multiple formats

Glutathione S-transferase and polyhistidine are the most commonly used protein tags for affinity purification. GST produces cleaner purifications than histidine tags and stabilizes overexpressed fusion proteins. GST binds specifically to reduced GSH in near-neutral, nondenaturing conditions. The bound GST-tagged protein is easily dissociated (eluted) from GSH resin by competitive displacement with a buffer containing free, reduced GSH. Alternatively, if the fusion protein is designed with a protease cleavage site, it may be released (cleaved) from the column at the GST tag junction using thrombin, HRV 3C protease or Factor Xa.

The Pierce Glutathione Agarose is highly effective for purifying a variety of GST-fusion proteins (Figure 6). The purity and yield of GST-fusion protein recovery is comparable to or better than glutathione resins from other suppliers (Figure 7). The resin is composed of 6% crosslinked beaded agarose, making it a structurally stable and resilient affinity support that enables repeated uses. The Pierce Glutathione Agarose is also amenable to chromatography cartridge format, producing baseline resolution and a sharp elution peak (Figure 8) with no visible resin bed compression.

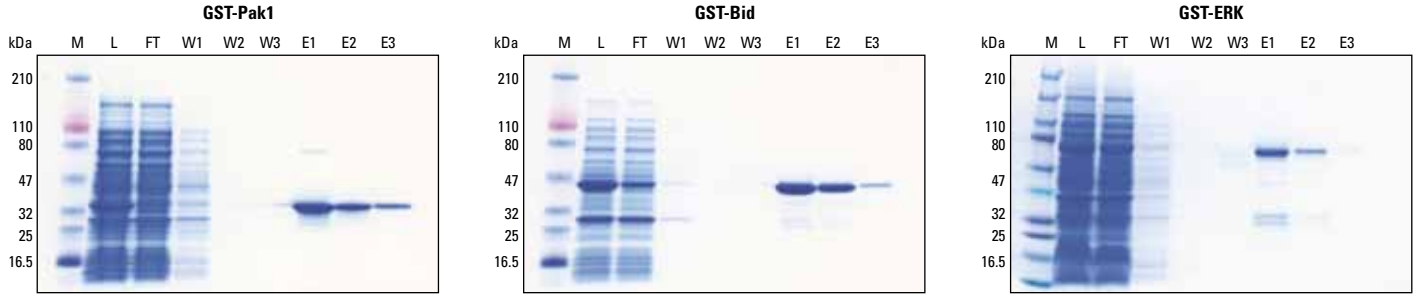
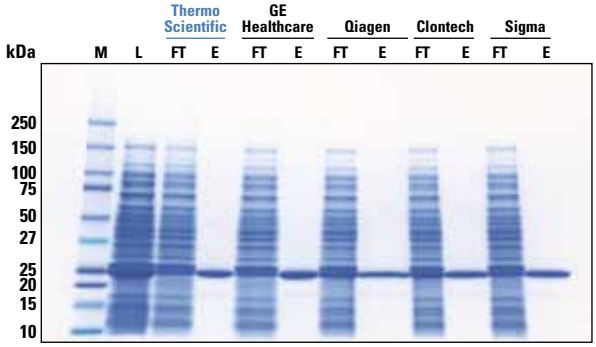


Figure 6. The Thermo Scientific Pierce Glutathione Agarose effectively purifies different GST-fusion proteins. Three GST-fusion proteins were expressed in *E. coli* and extracted with Thermo Scientific B-PER Reagent with Enzymes (Product # 90078). GST-Pak1 and GST-Bid were purified using the spin format, and GST-ERK was purified by gravity flow. GST-Pak1 and GST-Bid, 2.4mg and 11mg of total protein, respectively, were purified with a 0.2mL Pierce Glutathione Spin Column (Product # 16106) per kit instructions. For GST-ERK, 11.2mg of total lysate protein in buffer (50mM Tris, 150mM NaCl; pH 8.0) was applied to a 0.5mL bed of Pierce Glutathione Agarose (Product #16100) in a drip column and eluted with 125mM Tris, 150mM NaCl, 10mM glutathione at pH 8.0. Chromatography fractions were separated by SDS-PAGE and stained with Thermo Scientific GelCode Blue Stain Reagent (Product # 24590). **M =** Molecular weight markers; **L =** Lysate load; **FT =** Flow-through; **W =** Wash; **E =** Elution.



Vendor	Thermo Scientific	GE Healthcare	Qiagen	Clontech	Sigma
Yield	537µg	562µg	285µg	299µg	410µg
Purity	93%	93%	90%	91%	94%

Figure 7. Thermo Scientific Pierce Glutathione Agarose delivers high yield and high-purity GST-fusion proteins. *E. coli lysate* (14.4mg total protein) containing an overexpressed GST-fusion protein was incubated with 50µL GSH resin from various suppliers and purified per manufacturers' instructions. The amount of GST eluted from the resin (yield) was quantified by Thermo Scientific Coomassie Plus Protein Assay. Purity was assessed by densitometry of the stained gel lanes. **M=** Molecular weight markers; **L=** Lysate load; **FT=** Flow-through; **E=** Elution.

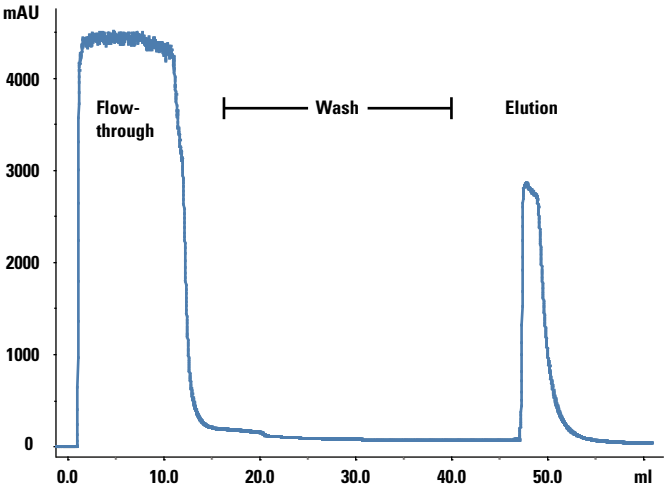


Figure 8. The glutathione chromatography cartridges provide an automatable solution for GST-fusion protein purification. Clarified *E. coli* lysate (50mg) containing overexpressed GST-Pak1 (34kDa) was applied to a 1mL glutathione chromatography cartridge in buffer (50mM Tris, 150mM sodium chloride; pH 8.0) at a flow rate of 0.5mL/minute using an ÄKTApurifier® System. The cartridge was washed with the same buffer at 1mL/minute. GST-Pak1 was eluted (50mM Tris, 150mM sodium chloride, 10mM glutathione; pH 8.0) at 1mL/minute.

Ordering Information

See catalog or www.thermoscientific.com/pierce for a complete description of products and kits.

Product #	Description	Pkg. Size
16100	Pierce Glutathione Agarose	10mL
16101	Pierce Glutathione Agarose	100mL
16102	Pierce Glutathione Agarose	500mL
16103	Pierce Glutathione Spin Columns, 0.2mL	25 columns
16104	Pierce Glutathione Spin Columns, 1mL	5 columns
16105	Pierce Glutathione Spin Columns, 3mL	5 columns
16106	Pierce GST Spin Purification Kit, 0.2mL	25-column kit
16107	Pierce GST Spin Purification Kit, 1mL	5-column kit
16108	Pierce GST Spin Purification Kit, 3mL	5-column kit
16109	Pierce Glutathione Chromatography Cartridges, 1mL	5 cartridges
16110	Pierce Glutathione Chromatography Cartridges, 5mL	2 cartridges

GST- and PolyHis-Tagged Pull-Down Assay Kits

Prepare to discover a new protein:protein interaction with your GST- or polyHis-tagged bait protein.

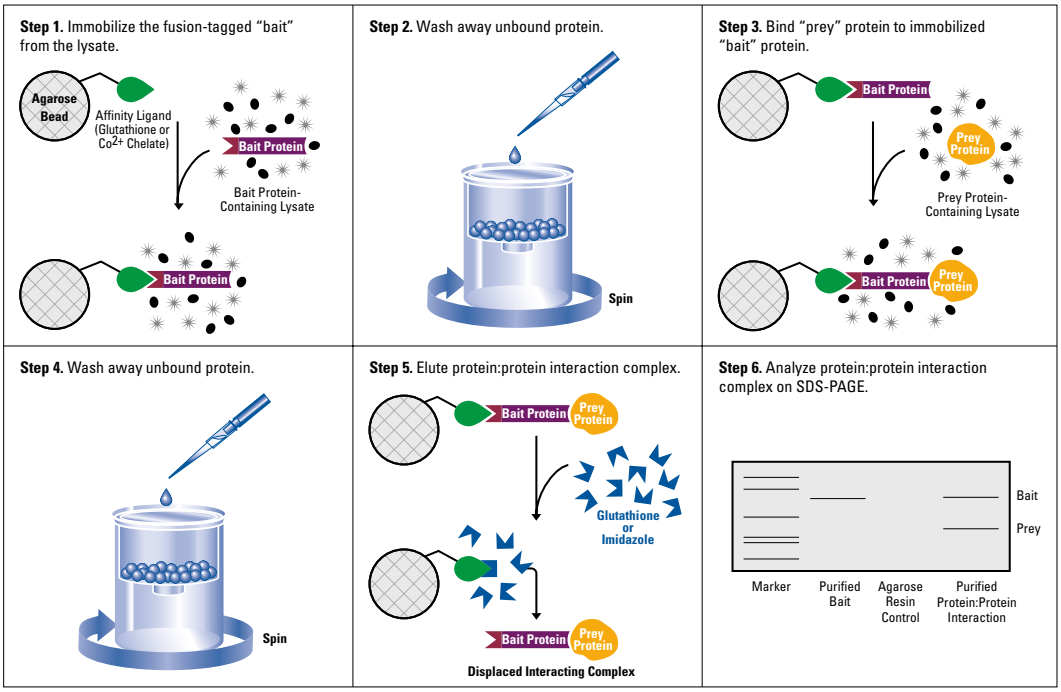
Identifying and characterizing the interactions of a given protein has emerged as the most valuable information that can be developed in the post-genomic era. Thermo Scientific Pierce Pull-Down Kits contain the necessary components to capture interacting proteins using the popular pull-down method. The only item you provide is an appropriately tagged fusion protein as the "bait." Our Pull-Down Kits are designed to teach the method to the first-time user and to shorten the time to a result for those experienced in this method.

Highlights:

- Provides a complete, affordable and easy-to-use strategy for discovery of protein:protein interactions
- Uses common laboratory equipment (e.g., microcentrifuges and mini-gels)
- Adapts to single- or multiple-sample demands
- Supplied complete with cell lysis buffer
- Flexible protocol aids in the capture of weak or transient interactions
- Efficient recovery of interacting complexes

Applications:

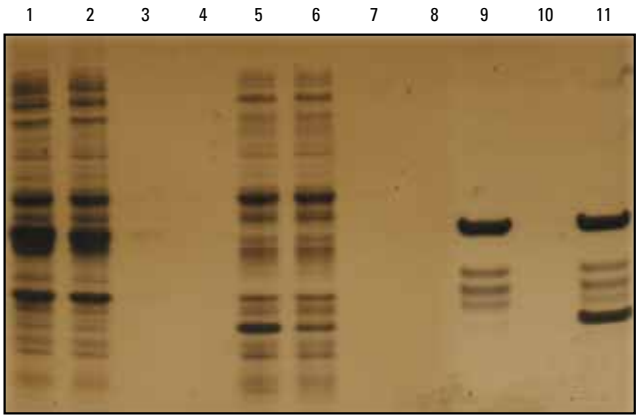
- Discover a new protein:protein interaction from a cell lysate
- Confirm a putative interaction from a cell lysate or with a previously purified protein
- Extract protein:protein interaction information from *in vitro* transcription/translation lysates



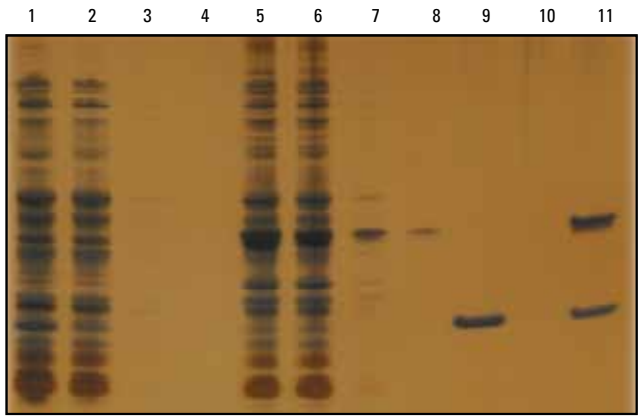
= Fusion Tag (GST or polyHis)

Generalized scheme for use of a Thermo Scientific Pierce Pull-Down Protein:Protein Interaction Kit using a GST-tagged or PolyHis-tagged protein as the "bait."

A.



B.



Lane #	A. GST-Tag Pull-Down	B. PolyHis-Tag Pull-Down
1	Lysate from *E. coli* expressing GST-tagged BIR2 **(bait protein).**	Lysate from *E. coli* expressing 9xHis-tagged wild-type Smac **(bait protein).**
2	Flow-through from the lysate in Lane 1 bound to an immobilized reduced glutathione support for 1 hour at 4°C.	Flow-through from the lysate in Lane 1 bound to an immobilized cobalt chelate support for 1 hour at 4°C.
3	Wash #1 of the support.	Wash #1 of the support.
4	Wash #2 of the support. (Washes 3-5 not shown.)	Wash #2 of the support. (Washes 3-5 not shown.)
5	Lysate from *E. coli* expressing 9xHis-tagged wild-type Smac **(prey protein).**	Lysate from *E. coli* expressing GST-tagged BIR2 **(prey protein).**
6	Flow-through from the lysate in Lane 5 is allowed to interact with the immobilized bait for 1 hour at 4°C.	Flow-through from the lysate in Lane 5 is allowed to interact with the immobilized bait for 1 hour at 4°C.
7	Wash #1 of the support.	Wash #1 of the support.
8	Wash #2 of the support. (Washes 3-5 not shown.)	Wash #2 of the support. (Washes 3-5 not shown.)
9	Bait control. Bait treated as described in Lanes 1-8 and subsequently eluted. No prey added – just binding buffer.	Bait control. Bait treated as described in Lanes 1-8 and subsequently eluted. No prey added – just binding buffer.
10	Prey control. Prey treated as described in Lanes 1-8 and subsequently eluted. No bait added – just binding buffer.	Prey control. Prey treated as described in Lanes 1-8 and subsequently eluted. No bait added – just binding buffer.
11	Elution of bait:prey complex (prepared in Lanes 1-8) from the support with 100mm reduced glutathione. Western blotting confirms that the minor bands observed in Lanes 9 and 11 are degradation products of GST-tagged BIR2.	Elution of bait:prey complex (prepared in Lanes 1-8) from the support with 250mm imidazole.

Validation of the Thermo Scientific Pierce Pull-Down Protein Interaction Kits using a known interacting pair.

Ordering Information

Product #	Description	Pkg. Size
21516	**Pierce Pull-Down GST Protein Interaction Kit** *Sufficient materials for performing 25 pull-down assays using a GST-tagged protein as the bait.*	Kit
	Includes: Immobilized Glutathione	750µL settled gel
	Lysis Buffer	250mL
	Glutathione	1g
	BupH Tris Buffered Saline	1 pack (500µL)
	Spin Columns Accessory Pack	27 columns
	Collection Tubes and Caps Accessory Pack	100 tubes

Acknowledgment
We gratefully acknowledge Dr. Yigong Shi of Princeton University for supplying the GST-BIR2- and 9xHis Smac/DIABIO-expressing clones. Dr. Shi's laboratory is engaged in research aimed at understanding the structural and molecular mechanisms involved in tumorigenesis and apoptosis.

Ordering Information

Product #	Description	Pkg. Size
21277	**Pierce Pull-Down PolyHis Protein Interaction Kit** *Sufficient materials for performing 25 pull-down assays using a polyhistidine-tagged protein as the bait.*	Kit
	Includes: Immobilized Glutathione	750µL settled gel
	Lysis Buffer	250mL
	4 M Imidazole Stock Solution	5mL
	BupH Tris Buffered Saline	1 pack (500µL)
	Spin Columns Accessory Pack	27 columns
	Collection Tubes and Caps Accessory Pack	100 tubes

References
1. Chai, J., *et al.* (2000). *Nature* **406**, 855-862.
2. Kaelin, W.G., *et al.* (1991). *Cell* **64**, 521-532.
3. Sambrook, J. and Russell, D.W. (2001). *Molecular Cloning: A Laboratory Manual 3rd Edition.* Chapter 18: Protein Interaction Technologies, Protocol #3: Detection of Protein-Protein Interactions using the GST Fusion Protein Pull-Down Technique. Cold Spring Harbor Laboratory Press.
4. Soutoglou, E., *et al.* (2000). *Mol. Cell* **5**, 745-751.

Covalent Coupling of Affinity Ligands to Chromatography Supports

Covalent Immobilization of Ligands

Affinity chromatography uses the specific interactions between two molecules for the purification of a target molecule. In practice, a ligand having affinity for a target molecule is covalently attached to an insoluble support and functions as bait for capturing the target from complex solutions.

The affinity ligand can be virtually any molecule that can specifically bind the target without displaying significant nonspecific binding toward other molecules in the solution. Ligands that have been used for affinity separations include small organic compounds that are able to dock into binding sites on proteins, inorganic metals that form coordination complexes with certain amino acids in proteins, hydrophobic molecules that can bind nonpolar pockets in biomolecules, proteins with specific binding regions that are able to interact with other proteins, and antibodies, which can be designed to target any biomolecule through their antigen-binding sites.

The concept of using immobilized affinity ligands to target biomolecules has extended beyond chromatographic applications. Affinity ligands are now coupled to latex beads, nanoparticles, macro-beads, membranes, microplates, array surfaces, dipsticks and a host of other devices that facilitate the capture of specific biomolecules. The application of affinity targeting includes purification, scavenging (or removal of contaminants), catalysis (or modification of target molecules) and a broad range of analytical uses to quantify a target molecule in a sample solution.

Designing custom affinity supports that are able to target unique biomolecules requires methods to covalently link a ligand to an insoluble matrix. Regardless of the intended application, the chemical reactions that make ligand attachment possible are well characterized and facilitate the attachment of biomolecules through their common chemical groups. The types of functionalities generally used for attachment include easily reactive components such as primary amines, sulfhydryls, aldehydes, carboxylic acids, hydroxyls, phenolic groups and histidinyl residues. Usually, the solid-phase matrix first is activated with a compound that is reactive toward one or more of these functional groups. The activated complex then can form a covalent linkage between the ligand and the support, resulting in ligand immobilization.

The type of linkage that is formed between the matrix and the immobilized ligand affects the performance of the affinity support in a number of ways. A linkage that allows the coupled ligand to leach from the matrix will result in contamination of the purified protein and shorten the useful life of the affinity support. A linkage that introduces a charged functional group into the support can cause nonspecific binding by promoting ion-exchange effects. A linkage that alters the structure of the matrix can change the flow and binding characteristics of the support. Cyanogen bromide (CNBr)-activated supports are informative as an example of these principles. This popular immobilization method results in a linkage that:

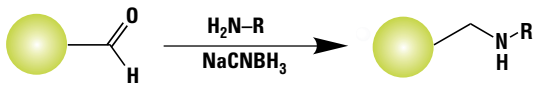
1. Has a constant leakage of ligand from the matrix that becomes a contaminant in the purified preparation.
2. Includes a charged isourea group in the linkage, resulting in nonspecific binding.
3. Causes extensive crosslinking of the matrix, reducing the ability of large molecules to penetrate into the interior of the resin.

We offer a number of activated affinity supports that are designed to couple ligands of every type via stable, uncharged covalent linkages that avoid introducing undesirable properties into the supports. The activation chemistry and protocols have been optimized to ensure excellent coupling yields with minimal effort under a variety of conditions. Each activated support comes with instructions for use and literature references as examples. The associated kits contain all the coupling buffers, wash buffers and columns necessary to perform the ligand immobilization and produce a support ready to perform an affinity separation.

Coupling Affinity Ligands through Amine Groups

The most common functional target for immobilizing protein molecules is the amine group, which is present on the vast majority of proteins because of the abundance of lysine side chain ε-amines and N-terminal α-amines. Thermo Scientific AminoLink Coupling Resin and AminoLink Plus Coupling Resin are prepared from crosslinked agarose supports, and they are designed to create a stable linkage between amine groups and the support material. AminoLink® Resins are activated to contain numerous aldehyde groups, which can be used to immobilize amine-containing ligands by reductive amination.

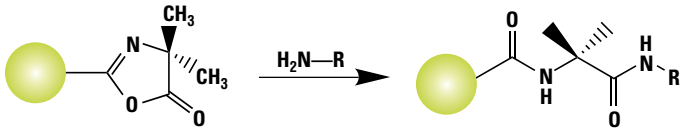
The immobilization reaction using reductive amination involves the formation of an initial Schiff base between the aldehyde and amine groups, which then is reduced to a secondary amine by the addition of sodium cyanoborohydride. The cyanoborohydride reducing agent used during the coupling process is mild enough not to cleave disulfides in most proteins, and it will not reduce the aldehyde reactants – only the Schiff base intermediates. It is best to avoid stronger reducing agents such as sodium borohydride because of the potential for disulfide reduction of the protein and reduction of the aldehydes on the support to hydroxyls, effectively quenching the reaction. Depending on the type and amount of ligand present, a coupling reaction using reductive amination can achieve immobilization yields of greater than 85%.



Aldehyde Group on Thermo Scientific AminoLink Coupling Resin

Affinity Ligand Coupled via Secondary Amine Bond

Another amine-reactive strategy that can be used for immobilization is the azlactone ring present in UltraLink Biosupport. A primary amine will react with an azlactone group in a ring-opening process that produces an amide bond at the end of a five-atom spacer. The group is spontaneously reactive with amines, requiring no additives or catalysts to drive the coupling process. The UltraLink Biosupport is supplied dry to ensure stability of the azlactone groups until use. Adding a quantity of the support to a sample containing a protein or other amine-containing molecule causes immobilization to occur within about one hour. For protein immobilization at high yield, it is recommended that the coupling buffer contain a lyotropic salt, which functions to drive the protein molecules toward the bead surface. This brings the hydrophilic amines close enough to the azlactone rings to react quickly. The simple nature of coupling affinity ligands to the UltraLink Biosupport along with its inherently low nonspecific binding makes it one of the best choices for immobilization.



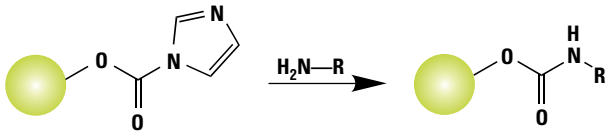
Azlactone Group on Thermo Scientific UltraLink Biosupport

Affinity Ligand Coupled via Amide Bond

Thermo Scientific UltraLink Biosupport binding capacity for various proteins.

Capacity	Protein	Coupling Buffer
35.0mg/mL	Myoglobin	0.1M CHES, 1.0M sodium citrate, pH 9.0
21.5mg/mL	Penicillin Acylase	0.1M sodium phosphate, 1.1M sodium sulfate, pH 7.4
20.9mg/mL	α-chymotrypsin	0.1M borate, 1.5 sodium sulfate, pH 9.0
35.5mg/mL	BSA	0.1M borate, 1.5 sodium sulfate, pH 9.0
29.8mg/mL	Lysozyme	0.1M borate, 1.0 sodium sulfate, pH 9.0
21.0mg/mL	Human IgG	0.1M borate, 1.5 sodium sulfate, pH 9.0

A third option for immobilizing amine-containing affinity ligands is the use of carbonyl diimidazole (CDI) to activate hydroxyls on agarose supports to form reactive imidazole carbamates. This reactive group is formed on the support in organic solvent and stored as a suspension in acetone to prevent hydrolysis. Reaction of the support in an aqueous coupling buffer with primary amine-containing ligands causes loss of the imidazole groups and formation of carbamate linkages. The coupling process occurs at basic pH (8.5–10), but it is a slower reaction with proteins than reductive amination or azlactone coupling. Thermo Scientific Pierce CDI Supports are available with the CDI-activated group, and they are particularly adept at immobilizing peptides and small organic molecules. The reaction also can be done in organic solvent to permit coupling of water-insoluble ligands.



Reactive Imidazole Carbamate on Thermo Scientific Pierce CDI Supports

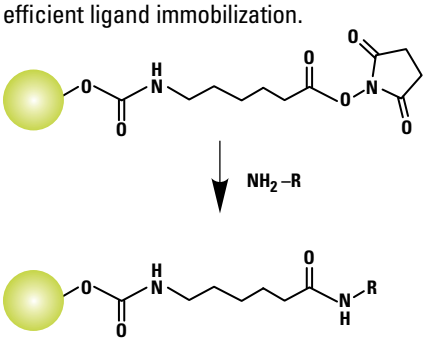
Affinity Ligand Coupled via Carbamate Bond

NHS-Activated Agarose resin uses safe, reliable NHS-ester chemistry and does not require hazardous chemicals for immobilization. Other amine-reactive supports, such as periodate-oxidized resins, use toxic sodium cyanoborohydride to stabilize the reaction linkage to primary amines and take 4 to 6 hours to complete. Traditional methods such as cyanogen bromide-activated supports also couple amines; however, this chemistry results in nonspecific binding and constant slow leakage of the coupled ligand. The Pierce NHS-Activated Agarose uses the stable NHS-ester chemistry that allows immobilization reactions to be completed in less than 1 hour.

The NHS-Activated Agarose coupling reaction is performed in an amine-free buffer at pH 7-9. Protein coupling efficiency is typically greater than 80%, regardless of the ligand's molecular weight or pI. Once a ligand is immobilized, the prepared resin can be used for multiple affinity purification procedures. The crosslinked beaded agarose has fast linear flow potential, making it useful for gravity-flow and low- to medium-pressure applications.

Covalent Coupling of Affinity Ligands to Chromatography Supports

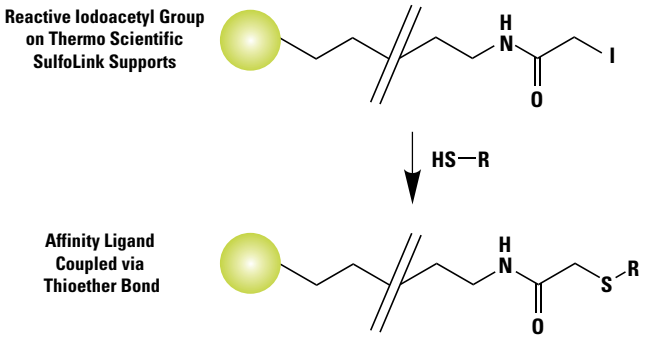
NHS-Activated Agarose resin is available in an anhydrous acetone slurry and as dry powder. The unique dry form does not require storage in or removal and disposal of the acetone solvent. In addition, the dry resin is ideal for coupling reactions with dilute samples because it concentrates the sample as the resin swells, reducing the volume of the starting material and resulting in highly efficient ligand immobilization.



Coupling Affinity Ligands through Sulfhydryl Groups

It is often advantageous to immobilize affinity ligands through functional groups other than just amines. In particular, the thiol group can be used to direct coupling reactions away from active centers or binding sites on certain protein molecules. Because amines occur at many positions on a protein's surface, it is usually difficult to predict where an amine-targeted coupling reaction will occur. However, if sulfhydryl groups that typically are present in fewer numbers are targeted for immobilization, then coupling can be done at discrete sites in a protein or peptide. Thiol groups (sulfhydryls) may be indigenous within a protein molecule or they can be added through the reduction of disulfides or through the use of various thiolation reagents. Sulfhydryls also can be added to peptide affinity ligands at the time of peptide synthesis by adding a cysteine residue at one end of the molecule. This ensures that every peptide molecule will be oriented on the support in the same way after immobilization.

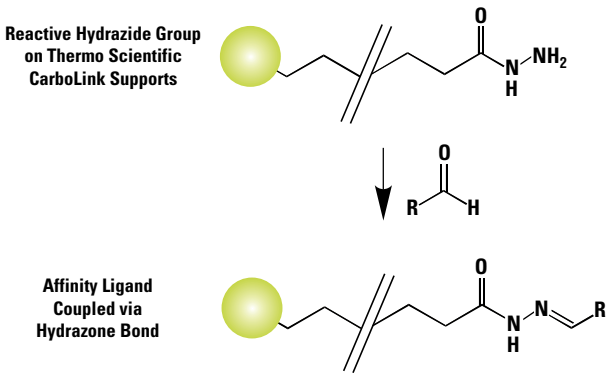
Thermo Scientific SulfoLink Coupling Resin is designed to efficiently react with thiol-containing molecules and immobilize them through a thioether linkage. The support contains an iodoacetyl group at the end of a long spacer arm, which reacts with sulfhydryls through displacement of the iodine. Optimal conditions for the reaction are an aqueous environment at slightly basic pH, wherein amines are not very reactive toward the iodoacetyl function, but thiols are highly reactive due to their increased nucleophilicity. The thioether bond that is formed provides a stable linkage to any sulfhydryl-containing molecule.



Coupling Affinity Ligands through Carbonyl Groups

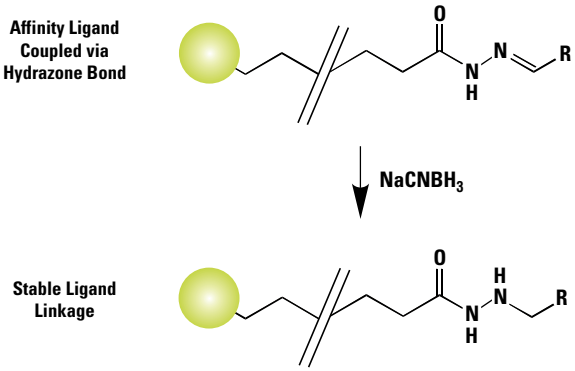
Most biological molecules do not contain carbonyl ketones or aldehydes in their native state. However, it might be useful to create such groups on proteins to form a site for immobilization that directs covalent coupling away from active centers or binding sites. Glycoconjugates, such as glycoproteins or glycolipids, usually contain sugar residues that have hydroxyls on adjacent carbon atoms, which can be periodate-oxidized to create aldehydes. Controlled oxidation using 1mm sodium meta-periodate at 0°C will selectively oxidize sialic acid groups to form an aldehyde functionality on each sugar. Using higher concentrations of periodate (10mm) at room temperature will result in oxidation of other sugar diols to create additional formyl groups. Aldehydes on the carbohydrate portion of glycoconjugates can be covalently linked with affinity supports through an immobilized hydrazide, hydrazine or amine group by Schiff base formation or reductive amination.

Thermo Scientific CarboLink Coupling Resin contains long spacer arms that terminate in hydrazide groups. Reaction of the hydrazides with aldehydes forms hydrazone linkages, which are a form of Schiff base displaying better stability than those formed between an amine and an aldehyde. The CarboLink™ Resin can be used to immobilize glycoproteins, such as antibodies, after periodate oxidation of the carbohydrate. Coupling antibodies in this manner specifically targets the heavy chains in the Fc portion of the molecule. Since this is away from the antigen-binding sites at the end of the Fv regions, immobilization using this route often results in the best retention of antigen-binding activity.



The CarboLink Resin also can be used to couple carbohydrates and sugars through their reducing ends. Aldehyde- or ketone-containing sugars will react with the immobilized hydrazide groups without oxidation of other sugar hydroxyls. However, this reaction may be dramatically slower than coupling with oxidized sugars because these native aldehydes or ketones are usually tied up in acetal or ketal ring structures. These rings can open in aqueous solution to reveal the aldehyde or ketone, but the open structure is present only a small percentage of the time. Thus, the reducing ends of sugars have decreased reactivity toward an immobilized hydrazide, sometimes requiring days of reaction time to obtain acceptable immobilization yields.

Although the hydrazone bond created between the immobilized hydrazide and an aldehyde is much more stable than amine-aldehyde Schiff bases, to obtain a leach-resistant linkage it is recommended that the Schiff base be reduced with sodium cyanoborohydride. This is especially true if a ligand is coupled that has only a single point of attachment to the support. Reduction of the hydrazone creates a stable bond that will perform well in affinity chromatography applications.

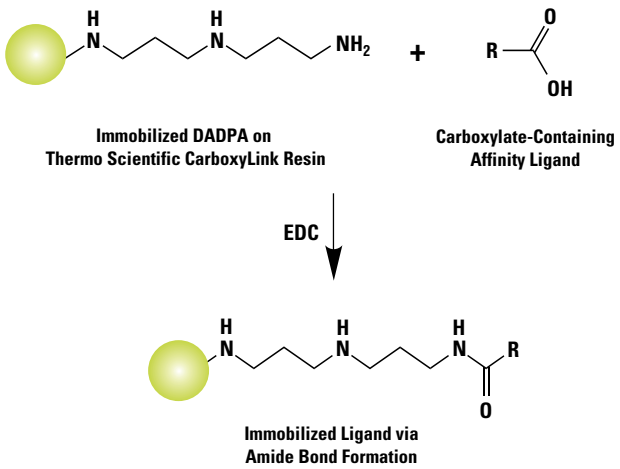


Coupling Affinity Ligands through Carboxyl Groups

The carboxyl group is a frequent constituent of many biological molecules. Particularly, proteins and peptides typically contain numerous carboxylic acids due to the presence of glutamic acid, aspartic acid and the C-terminal α-carboxylate group. Carboxylic acids may be used to immobilize biological molecules through the use of a carbodiimide-mediated reaction. Although no activated support contains a reactive group that is spontaneously reactive with carboxylates, chromatography supports containing amines (or hydrazides) may be used to form amide bonds with carboxylates. Molecules containing carboxylates may be activated to react with an immobilized amine (or hydrazide) through reaction with the water-soluble carbodiimide EDC.

EDC reacts with carboxylates to form an intermediate ester that is reactive with nucleophiles such as primary amines. The reaction takes place efficiently between about pH 4.5 and pH 7.5, and it is complete within two to four hours, depending on the temperature. The intermediate ester is subject to hydrolysis; therefore, it is beneficial if the amine-containing ligand to be immobilized is included in the reaction medium upon addition of EDC, so it can react immediately with the ester as it forms.

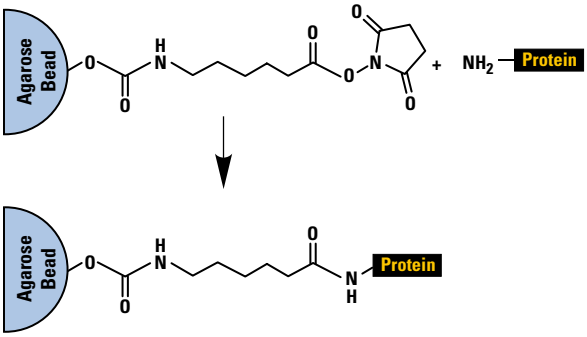
Thermo Scientific CarboxyLink Coupling Resin or the UltraLink DADPA Resin may be used to immobilize carboxylate-containing ligands by EDC. CarboxyLink® Resin contains a nine atom spacer arm and UltraLink DADPA contains a 12-atom spacer arm to minimize steric hindrance.



Products for Immobilizing Ligands through Primary Amines

Thermo Scientific Pierce NHS Ester-Activated Agarose

Pierce NHS-Activated Agarose Resin, available as a slurry or dry powder, allows for the simple and efficient immobilization of proteins to a beaded-agarose support. The activated agarose contains N-hydroxysuccinimide (NHS) ester with a spacer arm of at least 10 atoms in length that reacts with primary amines forming stable amide linkages. The NHS-Activated Agarose coupling reaction is performed in an amine-free buffer at pH 7-9, with typical coupling efficiencies of more than 85%. The prepared resin can be used for multiple affinity purification procedures. The crosslinked beaded agarose has fast linear flow potential, making it useful for gravity-flow and low- to medium-pressure applications.



Thermo Scientific NHS Ester-Activated Agarose Support immobilization chemistry.

Target: $-NH_2$
Support: 6% agarose
Binding capacity: >25mg protein/mL resin
Time: 30 minutes

Ordering Information

Product #	Description	Pkg. Size
26196	**Pierce NHS-Activated Agarose, Dry** Swell volume: 6-7.5mL/g of dry resin	1g
26197	**Pierce NHS-Activated Agarose, Dry** Swell volume: 6-7.5mL/g of dry resin	5g
26198	**Pierce NHS-Activated Agarose Spin Columns, 0.2mL** 25 spin columns containing 33mg of NHS-Activated Agarose Swell volume: 6- 7.5uL/mg of dry resin	25 columns
26199	**Pierce NHS-Activated Agarose Spin Columns, 2mL** 5 spin columns containing 330mg of NHS-Activated Agarose Swell volume: 6- 7.5uL/mg of dry resin	5 columns
26200	**Pierce NHS-Activated Agarose Slurry** 25mL of settled resin in anhydrous acetone	25mL

Thermo Scientific AminoLink Plus Immobilization Kits and Coupling Resin

The simplest and surest method for making an affinity purification resin with antibodies or other proteins.

AminoLink Plus Coupling Resin and Immobilization Kits use activated beaded agarose and a robust coupling chemistry to immobilize proteins and other ligands through primary amines (–NH_2) to the resin. Once an antibody or other ligand is immobilized, the prepared affinity resin can be used for a variety of purification methods involving batch or column chromatography. The resin and linkage are stable in binding and elution conditions typically used in affinity chromatography, enabling prepared resin to be used for at least 10 rounds of affinity purification.

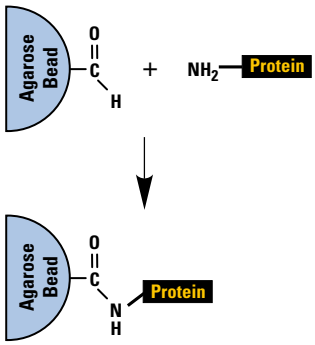
The AminoLink Plus Coupling Reaction involves spontaneous formation of Schiff base bonds between aldehydes (on the support) and amines (on the ligand) and their subsequent stabilization by incubation with a mild reductant (sodium cyanoborohydride; see more detailed reaction scheme on next page). The entire coupling reaction, called reductive amination, occurs in four to six hours in simple non-amine buffers such as PBS. Coupling efficiency with antibodies and typical proteins is generally greater than 85%, resulting in 1 to 20mg of immobilized protein per milliliter of agarose resin.

Highlights:

- **AminoLink Plus Coupling Resin** – aldehyde-activated crosslinked 4% beaded agarose
- **Ideal for antibodies and other proteins** – immobilize molecules via primary amines (–NH_2)
- **Flexible coupling conditions** – efficient (>85%) coupling over a wide range of pH (4–10) and buffer conditions (PBS or other non-amine buffer with or without organic solvent); regular (PBS, pH 7.2) and enhanced (borate, pH 10) coupling protocols provided
- **Stable, permanent immobilization** – coupling reaction results in stable, leak-resistant secondary amine bond between resin and ligand
- **Better than immobilization to CNBr-activated agarose** – bond is more stable and uncharged, resulting in less nonspecific binding in affinity purification procedures
- **Versatile and reusable** – prepared affinity resin is adaptable to column and batch affinity techniques and the resin is reusable for typical applications based on protein binding interactions
- **Convenient kits and product sizes** – choose one- or five-column kit containing complete sets of buffers, reagents and versatile spin/drip columns, or select bulk resin. Bulk quantities are available for manufacturing applications

Efficient immobilization of antibodies and other proteins. Percent coupling efficiency of different proteins to 2mL of Thermo Scientific AminoLink Plus Coupling Resin using the pH 7.2 coupling protocol.

Protein	Protein Applied (mg/mL)	Protein Coupled (mg/mL)	Percent Coupled
Protein G	4.6	4.0	83
Mouse IgG	4.7	4.5	96
Rat IgG	4.7	4.4	93
Goat IgG	0.9	0.8	84
Human IgG	4.8	4.6	97
Human IgM	0.9	0.8	93



Thermo Scientific AminoLink Support immobilization chemistry.

Target: –NH_2
Support: Highly crosslinked 4% agarose
Binding capacity: 20mg protein/mL resin
Time: 4 hours

References

Beall, A., *et al.* (1999). *J. Biol. Chem.* **274(16),** 11344–11351.
Nakasato, Y.R., *et al.* (1999). *Clin. Chem.* **45,** 2150–2157.
Allan, B.B., *et al.* (2000). *Science* **289,** 444–448.
Lu, R., *et al.* (2000). *J. Neurochem.* **74,** 320–326

Ordering Information

Product #	Description	Pkg. Size
44894	**AminoLink Plus Immobilization Kit**	Kit
	Sufficient reagents for preparing five affinity columns.	
	Includes: AminoLink Column	5 x 2mL
	Phosphate Buffered Saline (PBS)	1 pack
	Citrate-Carbonate Buffer	1 pack
	Quenching Buffer	50mL
	Wash Solution	240mL
	Sodium Cyanoborohydride Solution (5 M)	0.5mL
	Column Accessories	
20394	**AminoLink Plus Immobilization Trial Kit**	Kit
	Sufficient reagents for preparing one affinity column.	
	Includes: AminoLink Column	1 x 2mL
	Phosphate Buffered Saline (PBS)	1 pack
	Citrate-Carbonate Buffer	1 pack
	Quenching Buffer	15mL
	Wash Solution	50mL
	Sodium Cyanoborohydride Solution (5 M)	0.5mL
	Column Accessories	
20475	**AminoLink Plus Micro Immobilization Kit**	Kit
	Sufficient reagents for 10 coupling reactions using 25–100µg of protein and 20 affinity purifications.	
	Includes: AminoLink Plus Spin Columns, each containing 400µL of 25% slurry	10 each
	Phosphate Buffered Saline	1 pack
	Quenching Buffer	60mL
	Sodium Cyanoborohydride Solution	0.5mL
	Wash Solution	25mL
	Elution Buffer	50mL
	Microcentrifuge Collection Tubes	200 each
20501	**AminoLink Plus Coupling Resin**	10mL
20505	**AminoLink Plus Coupling Resin**	50mL

Covalent Coupling of Affinity Ligands to Chromatography Supports

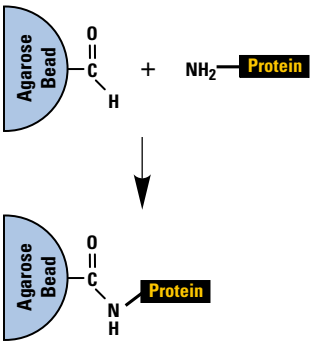
Thermo Scientific AminoLink Immobilization Kits and Coupling Resin

Links with primary amines (lysine residues and N*-terminus) on proteins, peptides, antigens or antibodies.*

AminoLink Coupling Resin is crosslinked 4% beaded agarose that has been activated with aldehyde groups. Proteins and other molecules with primary amines can be covalently attached (immobilized) to AminoLink Resin to make chromatography columns for use in affinity purification. The aldehyde groups form stable secondary amine bonds with primary amines such as exist in the side chain of lysine (K) residues, which are generally abundant and readily accessible in proteins. Once a protein is immobilized, the prepared affinity resin can be used for a variety of batch and column affinity purification methods involving binding interactions with the immobilized protein. The resin and linkage are stable in most binding and elution conditions typically used in affinity chromatography, enabling prepared resin to be used for multiple rounds of affinity purification procedures.

Highlights:

- **AminoLink Coupling Resin** – aldehyde-activated crosslinked 4% beaded agarose
- **Ideal for antibodies and other proteins** – immobilize molecules via primary amines (–NH_2)
- **Flexible coupling conditions** – efficient (>85%) coupling over a wide range of pH (4–10) and buffer conditions (PBS or other non-amine buffer with or without organic solvent)
- **Stable, permanent immobilization** – coupling reaction results in stable, leak-resistant secondary amine bond between resin and ligand
- **Better than immobilization to CNBr-activated agarose** – bond is more stable and uncharged, resulting in less nonspecific binding in affinity purification procedures
- **Versatile and reusable** – prepared affinity resin is adaptable to column and batch affinity techniques and the resin is reusable for typical applications based on protein binding interactions



Thermo Scientific AminoLink Support detailed immobilization chemistry.

Target: –NH_2
Support: 4% agarose
Binding capacity: 1-20mg protein/mL resin
Time: 4 hours

Efficient immobilization at a variety of pH values. The effect of coupling buffer pH on percent coupling efficiency of 9.58mg of human IgG to 2mL of Thermo Scientific AminoLink Resin using the standard coupling protocol.

pH	Coupling Efficiency of 9.58mg Human IgG
4	91.8%
5	92.7%
6	89.1%
7	87.3%
8*	85.3%
9*	94.9%
10*	98.4%

** Schiff-base formation occurs readily at high pH, but reduction to stable secondary amine bond requires subsequent incubation with sodium cyanoborohydride (AminoLink Reductant) at pH 7.2.*

References

Cheadle, C., *et al.* (1994). *J. Biol. Chem.* **269(39),** 24034–24039.
Cofano, F., *et al.* (1990). *J. Biol. Chem.* **265(7),** 4064–4071.
DeSilva, B.S. and Wilson, G.S. (1995). *J. Immunol. Method* **188,** 9–19.
Rivero-Lezcano, O.M., *et al.* (1994). *J. Biol. Chem.* **269(26),** 17363–17366.
Czermak, B.J., *et al.* (1999). *J. Immunol.* **162,** 2321–2325.
Assad, F.F., *et al.* (2001). *J. Cell Biol.* **152**, 531–543.
Zuk, P.A. and Elferink, L.A. (2000). *J. Biol. Chem.* **275(35)**, 26754–26764.6.

Ordering Information

Product #	Description	Pkg. Size
20381	**AminoLink Coupling Resin**	10mL
20382	**AminoLink Coupling Resin**	50mL
44890	**AminoLink Immobilization Kit** *Sufficient reagents to prepare five affinity columns. Each column can be used for up to 10 affinity purifications.* Includes: AminoLink Columns AminoLink Coupling Buffer Quenching Buffer Wash Solution, Sodium Cyanoborohydride Solution (5 M) Column Accessories	Kit 5 x 2mL 250mL 50mL 240mL 0.5mL
20384	**AminoLink Immobilization Trial Kit** Includes: AminoLink Column Reagents and Buffers	Trial Kit 1 x 2mL
44892	**AminoLink Reductant** (Sodium cyanoborohydride)	2 x 1g

Thermo Scientific UltraLink Biosupport

A durable, polyacrylamide resin, activated for efficient coupling of proteins.

UltraLink Biosupport is a durable, porous resin that is activated to enable efficient and direct covalent immobilization of proteins and other biomolecules through their primary amines for use in affinity purification procedures.

Highlights:

- **High coupling efficiency and capacity** – immobilizes proteins with very high efficiency and coupling capacity in 1 hour
- **Specific and leak-proof coupling chemistry** – reacts specifically with primary amines (–NH_2), resulting in amide bonds that are stable for use in many affinity purification procedures; coupling reaction has no leaving group to contaminate samples
- **Easy to use** – no pre-swelling or secondary reagents required; simply weigh the needed amount of dry support and add the ligand solution to initiate coupling reaction
- **Flexible coupling conditions** – perform immobilization reaction in any of a variety of non-amine buffers and pH levels; coupling is most efficient in buffers containing a lyotropic salt such as sodium citrate; coupling compatible with or without organic solvent
- **Excellent reusability** – prepared affinity resin can be used with typical binding and elution procedures for more than 100 cycles of affinity purification without significant loss of binding capacity
- **Durable, high-performance resin** – porous beads have a 60µm diameter, can withstand 100 psi (6.9 bar) and allow for linear flow-through rates of 3,000cm/hour

UltraLink Biosupport (Azlactone Ring on Bead) | Amine Ligand | Amide Bond Formation with Ring Opening

Thermo Scientific UltraLink Biosupport immobilization chemistry.

Target: –NH_2
Support: Polyacrylamide/azlactone copolymer
Binding capacity: >18mg protein/mL resin
Time: 4 hours

References

1. Ju, T., *et al.* (2002). *J. Biol. Chem.* **277,** 169–177.
2. Ju, T., *et al.* (2002). *J. Biol. Chem.* **277,** 178–186.
3. Kornfeld, R., *et al.* (1998). *J. Biol. Chem.* **273,** 23202–23210.
4. Liu, L.A. and Engvall, E. (1999). *J. Biol. Chem.* **274,** 38171–38176.

Ordering Information

Product #	Description	Pkg. Size
53110	UltraLink Biosupport (8-10mL)	1.25g
53111	UltraLink Biosupport (50mL)	6.25g
28388	BupH Citrate-Carbonate Buffer Packs	10 packs
28386	BupH Citrate-MOPS Buffer Packs	10 packs

Thermo Scientific Pierce CDI Supports

Carbonyldiimidazole-activated resins for ligand immobilization.

Highlights:

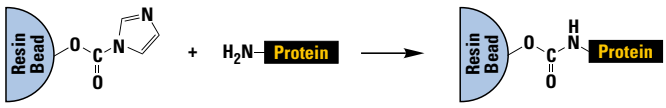
- **Reliable coupling chemistry** – immobilization occurs through the reaction of N-nucleophiles with 1,1′-carbonyldiimidazole groups of the resin to form a stable, uncharged N-alkylcarbamate linkages
- **Easy-to-use** – no secondary reagents needed; just wash equilibrate resin in alkaline coupling buffer and add ligand; reaction proceeds spontaneously
- **Stable activation** – half-life of hydrolysis is much longer than hydroxysuccinimide ester activations, making immobilization reactions simpler to prepare and control; simplifies filtration and washing before adding a ligand or protein
- **Well-defined coupling conditions** – reaction is most efficient with primary amines at pH 9-11

CDI-Activated Crosslinked 6% Beaded Agarose:

- Beaded agarose – the most popular resin for routine affinity purification methods
- Highly activated – at least 50µmol 1,1′-carbonyldiimidazole (CDI) groups per milliliter of resin
- Convenient form – supplied as stabilized, 50% slurry in acetone

CDI-Activated Trisacryl® GF-2000:

- Trisacryl resin – rigid, polyacrylamide matrix allows for high flow rates
- Hydrophilic matrix without charge effects – provides for low nonspecific binding
- Highly activated – at least 50µmol 1,1′ -carbonyldiimidazole (CDI) groups per milliliter of resin
- Convenient form – supplied as stabilized, 50% slurry in acetone



Imidazole Carbamate | Amine Ligand | Carbamate Linkage

CDI immobilization chemistry.

References

1. Shenoy, S.K., *et al.* (2001). *Science* **294,** 1307–1313.
2. Richardson, R.T., *et al.* (2000). *J. Biol. Chem.* **275,** 30378–30386.
3. Tanaka, M, *et al.* (2005). PLoS Biology **3**, 764–776.

Ordering Information

Product #	Description	Pkg. Size
20259	Pierce CDI (6X) Support 1,1′-Carbonyldiimidazole activated crosslinked 6% beaded agarose Supplied: stabilized in acetone slurry agarose hydrated particle size: 45–165µm Activation level: >50µmol/mL of resin	10mL
20377	Pierce CDI Trisacryl Support	50mL

Covalent Coupling of Affinity Ligands to Chromatography Supports

Products for Immobilizing Ligands through Sulfhydryl Groups

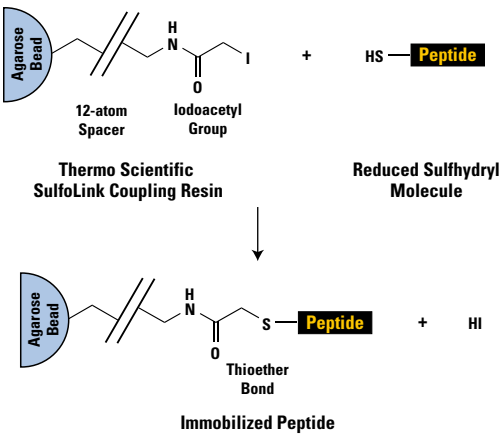
Thermo Scientific SulfoLink Immobilization Kits and Coupling Resin

Covalent immobilization of sulfhydryl-containing peptides or proteins for affinity purification.

SulfoLink Coupling Resin is porous, crosslinked 6% beaded agarose that has been activated with iodoacetyl groups. When incubated with a solution of peptide or protein that contains reduced cysteine residues, the iodoacetyl groups react specifically and efficiently with the exposed sulfhydryls (–SH) to form covalent and irreversible thioether bonds that permanently attach the peptide or protein to the resin. The result is a custom-made affinity resin for purification of antibodies, antigens and other molecules of interest.

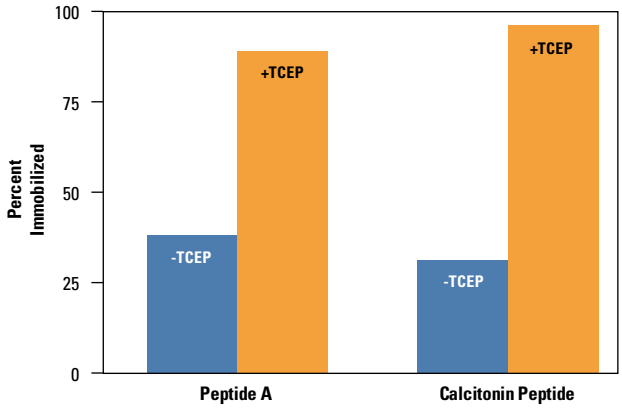
Highlights:

- **Specific conjugation through sulfhydryl (–SH) groups** – the iodoacetyl groups react specifically with sulhydryls to form irreversible thioether bonds
- **Separate kits optimized for peptides or proteins** – kits include optimized reagents for preparing peptide or protein samples for efficient immobilization
- **Fast** – spin columns increase protocol speed; prepare and couple samples in 2 hours (peptides) to 3.5 hours (proteins)
- **Flexible coupling conditions** – use pH 7.5–9.0 aqueous buffers, organic solvent (e.g., 20% DMSO) or denaturant (guanidine•HCl), as needed for protein or peptide solubility during coupling reaction
- **Easy-to-follow instructions** – streamLined protocols for sample preparation, immobilization, and affinity purification
- **High capacity** – immobilize 1–2mg peptide or 2–20mg protein per 2–mL column of SulfoLink Coupling Resin



Thermo Scientific SulfoLink Support immobilization chemistry.

Target: –SH
Support: 6% agarose or UltraLink® Resin
Binding capacity: 20mg protein/mL resin
Time: 2-3.5 hours



Improved retention of peptides on Thermo Scientific SulfoLink Resin with TCEP. TCEP effectively reduces peptides to maximize immobilization efficiency. Two peptides (Peptide A and human calcitonin peptide) were incubated with 25mm TCEP for 30 minutes and immobilized onto SulfoLink Resin via their reduced sulfhydryl groups. Peptide A's cysteine had oxidized during long-term storage and the calcitonin peptide contained an internal disulfide bond. Each peptide also contained an amine-terminal fluorescent probe by which the binding of the peptide could be monitored during the immobilization and wash steps.

References

Grunwald, R. and Meissner, G. (1995). *J. Biol. Chem.* **270(19)**, 11338–11347.
Seubert, P., *et al.* (1993). *Nature* **361**, 260–263.
Sukegawa, J., *et al.* (1995). *J. Biol. Chem.* **270(26)**, 15702–15706.
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Tan, M., *et al.* (2000). *Proc. Natl. Acad. Sci.* USA **97,** 109–114.
Quill, T.A., *et al.* (2001). *Proc. Natl. Acad. Sci. USA* **98,** 12527–12531.
Tokumaru, H., *et al.* (2001). *Cell* **104,** 421–432.
Assad, F.F., *et al.* (2001). *J. Cell Biol.* **152,** 531–543.

Ordering Information

Product #	Description	Pkg. Size
44995	**SulfoLink Immobilization Kit for Proteins**	Kit
	Sufficient reagents to prepare five affinity columns.	
	Kit contains: SulfoLink Columns	5 x 2mL
	SulfoLink Sample Preparation Buffer	7.5mL
	SulfoLink Coupling Buffer	500mL
	Wash Solution	120mL
	2-Mercaptoethylamine•HCl	5 x 6mg
	L-Cysteine•HCl	100mg
	Zeba Desalt Spin Columns	5 x 5mL
	Phosphate Buffered Saline	1 Pack
	Columns Accessories	
44999	**SulfoLink Immobilization Kit for Peptides**	Kit
	Sufficient reagents to prepare five affinity columns.	
	Kit contains: SulfoLink Columns	5 x 2mL
	SulfoLink Coupling Buffer	120mL
	Wash Solution	120mL
	Bond-Breaker TCEP Solution	0.5mL
	L-Cysteine•HCl	100mg
	Phosphate Buffered Saline	1 Pack
	Column Accessories	
20325	**SulfoLink Immobilization Trial Kit**	Kit
	Sufficient reagents to prepare 1 affinity column with a peptide or protein.	
	Kit contains: SulfoLink Column	1 x 2mL
	SulfoLink Sample Preparation Buffer	7.5mL
	SulfoLink Coupling Buffer	120mL
	Wash Solution	25mL
	2-Mercaptoethylamine•HCl	6mg
	Bond-Breaker TCEP Solution	0.5mL
	Cysteine•HCl	100mg
	Zeba Desalt Spin Column	5mL
	Phosphate Buffered Saline	1 Pack
	Columns Accessories	
20401	**SulfoLink Coupling Resin**	10mL
20402	**SulfoLink Coupling Resin**	50mL
20404	**SulfoLink Coupling Resin**	250mL

Thermo Scientific UltraLink Iodoacetyl Resin

Polyacrylamide resin for coupling sulfhydryl-containing ligands.

UltraLink Iodoacetyl Resin is a durable, porous resin that is activated to enable efficient and direct covalent immoblization of peptides and other ligands through their sulfhydryl groups (–SH) for use in affinity purification procedures. The beaded resin is a hydrophilic copolymer of polyacrylamide and azlactone having a rigid polymeric structure with high surface area and pore volume. Each azlactone group has been modified to form a 15-atom spacer arm that terminates in an iodoacetyl group, which is capable of reacting with sulfhydryl groups (e.g., side chain of reduced cysteine residues) to covalently immobilize peptide or other sulfhydryl-containing ligands. The bead structure and efficiently coupling chemistry of Iodoacetyl-Activated UltraLink Support results in high protein binding capacity, high linear flow rates, low nonspecific binding and overall superior performance in affinity chromatography. UltraLink Resins are ideal for medium pressure applications such as FPLC.

Highlights:

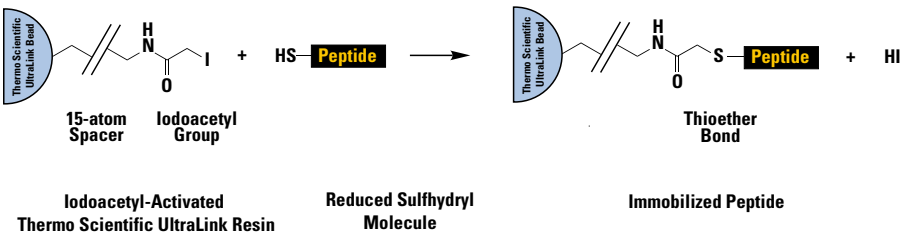
- **High coupling efficiency and capacity** – immobilizes sulfhydryl-containing proteins or other ligands with high efficiency and coupling capacity in 1 hour
- **Specific and leak-proof coupling chemistry** – reacts specifically with sulfhydryl groups (reduced thiols), resulting in thioether bonds that are stable for use in many affinity purification procedures
- **Simple coupling conditions** – perform immobilization reaction in any of a variety of buffers; coupling is most efficient and specific at pH 8.0–8.5; coupling compatible with or without organic solvent
- **Excellent reusability** – prepared affinity resin can be used with typical binding and elution procedures for many cycles of affnity purification without significant loss of binding capacity
- **Durable, high-performance resin** – porous beads have a 60µm diameter, can withstand 100 psi (6.9 bar) and allow for linear flow-through rates of 3,000cm/hour

References

1. Ju, T., *et al.* (2002). *J. Biol. Chem.* **277,** 169–177.
2. Hill, K., *et al.* (2000). *J. Biol. Chem.* **275(6),** 3741–3744.
3. Liu, L.A. and Engvall, E. (1999). *J. Biol. Chem.* **274,** 38171–38176.
4. Bicknell, A.B., *et al.* (2001). *Cell* **105,** 903–912.

Ordering Information

Product #	Description	Pkg. Size
53155	**UltraLink Iodoacetyl Resin** Support: UltraLink Biosupport	10mL



Thermo Scientific UtraLink Iodoacetyl immobilization chemistry.

Thermo Scientific UltraLink Iodoacetyl Micro Peptide Coupling Kit

Easily prepare a small-scale affinity column with sulfhydryl-containing peptides.

The Micro Peptide Coupling Kit is a microcentrifuge spin column kit for immobilizing small amounts (25–250µg) of sulfhydryl-containing peptides (e.g., cysteine-terminated peptides) onto a beaded porous resin to create a small, reusable affinity column. The coupling and affinity purification procedures are optimized for small sample volumes (200–300µL). Wash and elution steps are achieved rapidly and efficiently with the convenient microcentrifuge spin columns. Each kit contains sufficient reagents for 10 coupling reactions and 20 affinity purifications. The kit is ideal for immobilizing peptide antigens that containing a terminal cysteine residue for use in purifying specific antibodies from small serum, ascites or culture supernatant samples.

Highlights:

- **Optimized for small scale** – ideal for coupling small amounts (25–250µg) of sulfhydryl-containing peptide or protein for purification of specific antibodies from crude serum
- **High-performance affinity resin** – uses durable, polyacrylamide-based UltraLink Iodoacetyl Resin for specific reaction to sulfhydryl groups
- **Efficient coupling chemistry** – immobilization efficiency >85% (as measured with 1 hour reaction using insulin, calcitonin and osteocalcin peptides)
- **Fast and easy to use** – perform wash and elution steps using a microcentrifuge
- **Reusable** – use prepared peptide resin several times with no significant loss of capacity

Ordering Information

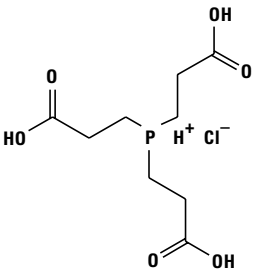
Product #	Description	Pkg. Size
20485	**Micro Peptide Coupling Kit** *Sufficient for coupling 10 sulfhydryl-containing peptides or proteins and perform 20 affinity purifications.*	Kit
	Kit contents: UltraLink Iodoacetyl Spin Columns, 0.1mL	10 columns
	Coupling Buffer	100mL
	L-Cysteine-HCl	100mg
	Wash Solution	25mL
	PBS Pack (makes 500mL)	1 pack
	IgG Elution Buffer	50mL
	Collection Tubes	200 tubes

Covalent Coupling of Affinity Ligands to Chromatography Supports

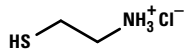
Thermo Scientific Disulfide Reducing Agents

Reduce disulfide bonds to produce sulfhydryl groups for immobilization on SulfoLink or UltraLink Resins.

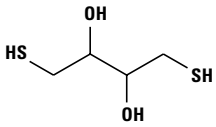
Free sulfhydryls are required for immobilization onto sulfhydryl-reactive affinity supports. Cysteines in proteins and peptides usually exist as cystines (disulfide bridges) and must be reduced to expose sulfhydryls for coupling. Reduction can be accomplished with free or immobilized reducing agents. Free reducing agents are efficient in reducing all disulfides in proteins, including those buried in the tertiary structure, but they must be removed from the reduced sample with a desalting column before coupling to the support. Immobilized reducing agents enable reduction of disulfides and simple removal of the reduced sample from the reducing agent. This is especially helpful when reducing peptides whose small size prevents them from being effectively desalted.



TCEP•HCl
MW 286.65



2-Mercaptoethylamine•HCl
MW 113.61



DTT
MW 154.25

Ordering Information

Product #	Description	Pkg. Size
20408	**2-Mercaptoethylamine•HCl**	6 x 6mg
20290	**DTT, Cleland's Reagent** (Dithiothreitol)	5g
20291	**Dithiothreitol (DTT) in No-Weigh™ Format** 7.7mg DTT/tube. Makes 100µL of 0.5 M DTT.	48 tubes
20490	**TCEP•HCl** (Tris[2-carboxyethyl]phosphine hydrochloride)	1g
20491	**TCEP•HCl**	10g
77720	**Bond-Breaker TCEP Solution, Neutral pH**	5mL
77712	**Immobilized TCEP Disulfide Reducing Gel**	5mL

Thermo Scientific Ellman's Reagent and Sulfhydryl Addition Kit

Measure and add free sulfhydryls to ensure success of cysteine-targeted immobilization.

Ellman's Reagent, also called DTNB, is a versatile water-soluble compound for quantifying free sulfhydryl groups in solution. A solution of this compound produces a measurable yellow-colored product when it reacts with sulfhydryls (–SH groups). By testing an unknown sample, such as a peptide having a terminal cysteine residue, compared to a standard curve made with known amounts of free, reduced cysteine (Product # 44889), availability of reduced sulfhydryls in the sample can be determined.

The Sulfhydryl Addition Kit provides the essential reagents and procedure for creating new sulfhydryl groups on a protein or other molecule that contains available primary amines ($–NH_2$). The kit uses SATA reagent, which forms covalent bonds to primary amines. The result is addition of a stable (capped) sulfhydryl group, which can later be exposed by gentle treatment with hydroxylamine, making the molecule ready for conjugation to SulfoLink Coupling Resin, UltraLink Iodoacetyl Resin or other sulfhydryl-reactive immobilization method.

Ordering Information

Product #	Description	Pkg. Size
23460	**Sulfhydryl Addition Kit** *Adds free sulfhydryl groups to proteins.*	Kit
	Includes: SATA	2mg
	Hydroxylamine•HCl	5mg
	10X Conjugation Buffer Stock	20mL
	Phosphate Buffered Saline Pack	1 pack
	Dimethylformamide (DMF)	1mL
	Dextran Desalting Column	1 x 5mL
	Column Extender	1
	Ellman's Reagent (DTNB)	2mg
	Cysteine•HCl H_2O	20mg
22582	**Ellman's Reagent** (5,5'-Dithio-bis-[2-nitrobenzoic acid])	5g
44889	**Cysteine•HCl**	5g

Products for Immobilizing Ligands through Carbonyl Groups

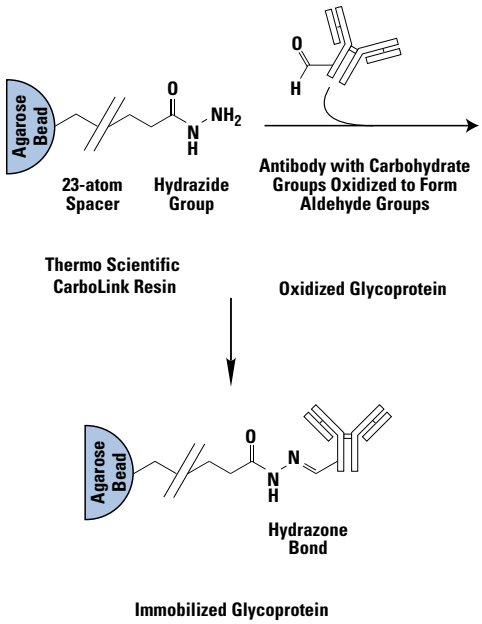
Thermo Scientific CarboLink Immobilization Kit and Coupling Resins

Immobilize polyclonal antibodies and other glycoproteins through carbohydrate groups.

CarboLink Coupling Resin and Kits provide for covalent immobilization of glycoproteins and other carbohydrate-containing molecules to beaded agarose (or polyacrylamide UltraLink Support) for use in affinity purification procedures. Carbohydrate moieties in glycoproteins contain common sugars whose cis-diol groups are easily oxidized with sodium *meta*-periodate (included in the CarboLink Kit) to yield aldehydes. When incubated with the CarboLink Resin, these aldehyde groups react spontaneously with the hydrazide group of the activated resin to form stable, covalent bonds. The immobilization strategy is especially useful for glycoproteins, such as polyclonal antibodies, because it allows attachment of the molecule at domains that will not interfere with binding sites that are critical for the intended affinity purification. Once a molecule is coupled, the prepared affinity resin can be used multiple times in typical protein affinity purification procedures.

Highlights:

- **CarboLink Coupling Resin** – hydrazide-activated crosslinked 6% beaded agarose (or hydrazide-activated UltraLink Support, a beaded, polyacrylamide resin)
- **Efficient immobilization** – couple 1–5mg of oxidized polyclonal antibody or other glycoprotein per milliliter of resin (CarboLink Resin contains greater than 14µmol hydrazide groups per milliliter)
- **Stable linkage** – resonance structure of the hydrazone bonds are sufficiently stable to allow multiple rounds of affinity purification with one batch of prepared resin; no stabilizing reductant required
- **Flexible and gentle coupling conditions** – immobilization reaction completed in simple buffers (PBS or other non-amine buffer with or without organic solvent) at near-neutral pH
- **Ideal for polyclonal antibodies** – immobilizes IgG through carbohydrates in the Fc region, so both antigen binding sites are free to interact with the antigen in the mobile phase
- **Effective for any molecule with oxidizable sugars** – first step is oxidation of the sugar groups, which allows the cis-diols of the IgG to be transformed into reactive aldehyde moieties; these aldehydes then combine with hydrazide groups on the matrix to form stable, leak-resistant linkages
- **Convenient kits and product sizes** – choose one- or five-column kit containing complete sets of buffers, oxidizing reagent and versatile spin/drip columns containing the beaded agarose resin; or choose the polyacrylamide-based UltraLink Support. Bulk quantities are available for manufacturing applications



Thermo Scientific CarboLink Support immobilization chemistry.

Target: –CHO
Support: 6% agarose
Binding capacity: 5mg protein/mL resin
Time: 8 hours

References

1. Kumar, P.G., *et al.* (2001). *J. Biol. Chem.* **276,** 41357–41364.
2. Strakova, Z., *et al.* (1997). *Mol. Pharmacol.* **51,** 217–224.
3. Brown, M.A., *et al.* (2000). *J. Biol. Chem.* **275,** 19795–19802.
4. Butko, P., *et al.* (1999). *J. Immunol.* **163,** 2761–2768.
5. Sequra, M., *et al.* (1999). *Infect. Immun.* **67(9),** 4646

Ordering Information

Product #	Description	Pkg. Size
20355	**CarboLink Immobilization Trial Kit**	Kit
	Kit contains: CarboLink Column	1 x 2mL
	CarboLink Coupling Buffer	60mL
	CarboLink Wash Solution	15mL
	Sodium meta periodate	1 x 5mg
	Zeba Desalting Column	1 x 5mL
44910	**CarboLink Immobilization Kit**	Kit
	Kit contains: CarboLink Columns	5 x 2mL
	CarboLink Coupling Buffer	250mL
	CarboLink Wash Solution	100mL
	Sodium meta periodate	5 x 5mg
	Zeba Desalting Columns	5 x 5mL
20391	**CarboLink Coupling Resin**	10mL
53149	**UltraLink Hydrazide Resin**	10mL
20504	**Sodium *meta*-Periodate**	25g

Covalent Coupling of Affinity Ligands to Chromatography Supports

Products for Immobilizing Ligands through Carboxyl Groups

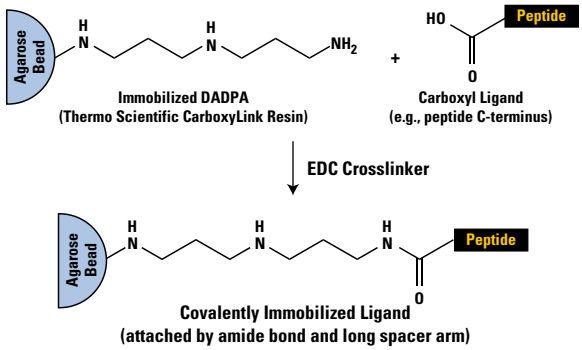
Thermo Scientific CarboxyLink Immobilization Kits and Coupling Resins

Immobilize peptides via carboxyl groups to create an affinity column.

CarboxyLink Coupling Resin and Kits provide for covalent immobilization of peptides or other carboxyl-containing (–COOH) molecules to a porous, beaded resin for use in affinity purification procedures. CarboxyLink Resin is crosslinked beaded agarose (or polyacrylamide UltraLink Support) that has been activated with diaminodipropylamine (DADPA) to contain long spacer arms, each with a primary amine at the end. When incubated with the resin and the carbodiimide crosslinker EDC (included in the CarboxyLink Immobilization Kit), carboxyl-containing molecules become permanently attached to the support by stable amide bonds. Once a molecule is coupled, the prepared affinity column can be used multiple times in typical protein affinity purification procedures. CarboxyLink Coupling Resins can also be used to immobilize other kinds of molecules using alternative amine-reactive crosslinking chemistries.

Highlights:

- **CarboxyLink Coupling Resin** – DADPA-activated crosslinked 4% beaded agarose (or DADPA-activated UltraLink Support, a beaded polyacrylamide resin)
- **Efficient immobilization** – couple 1–2mg of peptide per milliliter of resin (CarboxyLink Agarose Resin activated with greater than 16µmol amine milliliter of resin; DADPA on UltraLink Support activated with greater than 40µmol amine milliliter of resin)
- **Stable linkage** – immoblization results in covalent attachment of carboxyl groups by amide bonds, allowing for multiple rounds of affinity purification with one batch of prepared resin
- **Flexible and gentle coupling conditions** – immobilization reaction completed in simple MES or other non-amine and non-carboxyl, near-neutral buffer, with or without organic solvent.
- **Ideal for unmodified peptides** – immobilizes peptides with high capacity and various orientations without steric hindrance, allowing for effective use in affinity purification of specific antibodies
- **Convenient kits and product sizes** – choose five-column kits with complete sets of buffers, crosslinker and versatile spin/drip columns containing either type of resin (agarose or polyacrylamide) or choose stand-alone resin for other uses



Thermo Scientific CarboxyLink Support immobilization chemistry.

Target: –COOH
Support: 4% agarose or UltraLink® Resin
Binding capacity: 5mg protein/mL resin
Time: 4 hours

Reference

Yoo, B.C., *et al.* (2002). *J. Biol. Chem.* **277,** 15325–15332.

Ordering Information

Product #	Description	Pkg. Size
44899	**CarboxyLink Immobilization Kit** Kit contains: CarboxyLink Columns (DADPA agarose) EDC Crosslinker Coupling Buffer (MES-buffered Saline) Wash Solution (1 M NaCl) Column Accessories	Kit 5 x 2mL 5 x 60mg 500mL 120mL
20266	**CarboxyLink Coupling Resin** Support: Crosslinked 4% beaded agarose Loading: 16–20µmol available amino groups/mL of resin	25mL
53154	**CarboxyLink Immobilization Kit with UltraLink Support** Kit contains: DADPA UltraLink Columns EDC Crosslinker Coupling Buffer (MES-buffered Saline) Wash Solution (1 M NaCl) Column Accessories	Kit 5 x 2mL 5 x 60mg 500mL 120mL
22980	**EDC** 1-Ethyl-3-[3-dimethylaminopropyl]carbodiimide hydrochloride	5g
28390	**BupH MES Buffered Saline Packs**	10 pack

Antibody immobilization: choosing the best Thermo Scientific Support.

	AminoLink Plus Coupling Resin	UltraLink Biosupport	CarboLink Coupling Resins	SulfoLink Coupling Resins	Crosslink IP Kits See page 41
Monoclonal Antibodies	**Advantages:** • Good choice when only small amounts of antibody are available • Couple over a broad pH range • Good coupling efficiency **Disadvantages:** • Reduction of Schiff's base with sodium cyanoborohydride may adversely affect monoclonals • Some antibodies may be coupled through antigen-binding site	**Advantages:** • Good choice if antibody can withstand 1.0M sodium citrate or sulfate • High capacity • Fast, efficient coupling • Good, for large-scale or fast-flow applications **Disadvantages:** • Some antibodies may be coupled through antigen-binding site • Some antibodies may precipitate in high-salt buffer	**Advantages:** • Correctly orients antibody • Antibody must be able to withstand oxidation conditions • Good for antibodies with low avidity for antigen **Disadvantages:** • Not all monoclonals have carbohydrate accessible for coupling • Conditions necessary for coupling may adversely affect some monoclonals	**Advantages:** • Good choice for antibodies that have extremely high avidity for their antigen • Allows for gentle elution conditions **Disadvantages:** • Must first reduce antibody prior to coupling • Not good for antibodies with low affinity for their antigens	**Advantages:** • Allows for correct orientation of antibodies • Gentle coupling conditions • Protein A/G will bind most antibodies **Disadvantages:** • If purifying antigen from serum, antibodies may bind to Protein A/G and co-purify with antigen • Crosslinking results in some loss of antibody activity
Polyclonal Antibodies	**Advantages:** • Excellent coupling efficiency • Good antigen recovery **Disadvantages:** • Some antibodies may be coupled through antigen-binding site	**Advantages:** • Good choice for large-scale or fast-flow applications • High capacity • Fast, efficient coupling **Disadvantages:** • Some antibodies may be coupled through antigen-binding site • Some antibodies may precipitate in high-salt buffer	**Advantages:** • Correctly orients antibody • Antibody must be able to withstand oxidation conditions • Good for antibodies with low avidity for antigen **Disadvantages:** • Conditions necessary for coupling may adversely affect some antibodies	**Advantages:** • Good choice for antibodies that have avidity for their antigen • Allows for gentle elution conditions **Disadvantages:** • Must first reduce antibody prior to coupling • Not good for antibodies with low affinity for their antigens	**Advantages:** • Allows for correct orientation of antibodies • Gentle couple conditions • Protein A/G will bind most antibodies **Disadvantages:** • If purifying antigen from serum, antibodies may bind to Protein A/G and co-purify with antigen • Crosslinking results in some loss of antibody activity
High-Activity Antibodies				**Advantages:** • Immobilization of reduced antibody allows for gentler elution conditions	
Low-Activity Antibodies			**Advantages:** • Correctly orients antibody **Disadvantages:** • Conditions necessary for coupling may adversely affect some monoclonals		**Advantages:** • Allows for correct orientation of antibodies • Gentle couple conditions • Protein A/G will bind most antibodies **Disadvantages:** • If purifying antigen from serum, antibodies may bind to Protein A/G and co-purify with antigen • Crosslinking results in some loss of antibody activity

Immunoprecipitation

The topic of co-immunoprecipitation (co-IP) is best preceded by a discussion of immunoprecipitation (IP) to help frame an understanding of the principles involved.

IP is one of the most widely used methods for antigen detection and purification. An important characteristic of IP reactions is their potential to deliver not only the target protein, but also other macromolecules that interact with the target.

The IP Principle

The principle of an IP is very simple (Figure 1). An antibody (monoclonal or polyclonal) against a specific target antigen is allowed to form an immune complex with that target in a sample, such as a cell lysate. The immune complex is then captured on a solid support to which either Protein A or Protein G has been immobilized (Protein A or Protein G binds to the antibody, which is bound to its antigen). The process of capturing this complex from the solution is referred to as precipitation. Any proteins not "precipitated" by the immobilized Protein A or Protein G support are washed away. Finally, components of the bound immune complex (both antigen and antibody) are eluted from the support and analyzed by SDS-PAGE (gel electrophoresis), often followed by Western blot detection to verify the identity of the antigen.

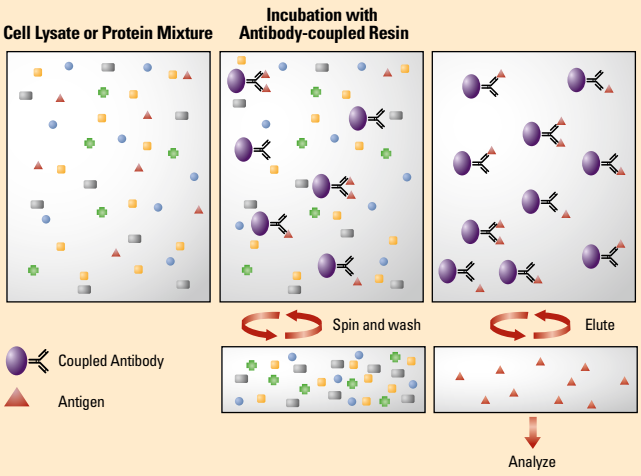


Figure 1. Summary of a traditional immunoprecipitation procedure.

Traditional immunoprecipitation involves the following steps:

1. Form the antigen-antibody complex (immune complex) by incubating specific antibody with the antigen-containing sample for 1 hour to several hours.
2. Capture the immune complex to Protein A or Protein G agarose beads by incubation for 0.5-2 hours.
3. Remove any non-bound protein (non-immune complex sample components) from the precipitated complex by washing beads with additional sample buffer.
4. Boil beads in reducing SDS-PAGE sample loading buffer.
5. Recover eluted sample in loading buffer and analyze by SDS-PAGE.
6. Perform Western blot analysis, probing with antigen-specific antibody.

Co-IP vs. IP

Co-IP is a popular technique for protein interaction discovery. Co-IP is conducted in essentially the same manner as IP (Figure 2). However, in a co-IP the target antigen precipitated by the antibody "co-precipitates" a binding partner/protein complex from a lysate; i.e., the interacting protein is bound to the target antigen, which becomes bound by the antibody that becomes captured on the Protein A or Protein G resin. The assumption usually made when associated proteins are co-precipitated is that these proteins are related to the function of the target antigen at the cellular level. This is only an assumption, however, that is subject to further verification.

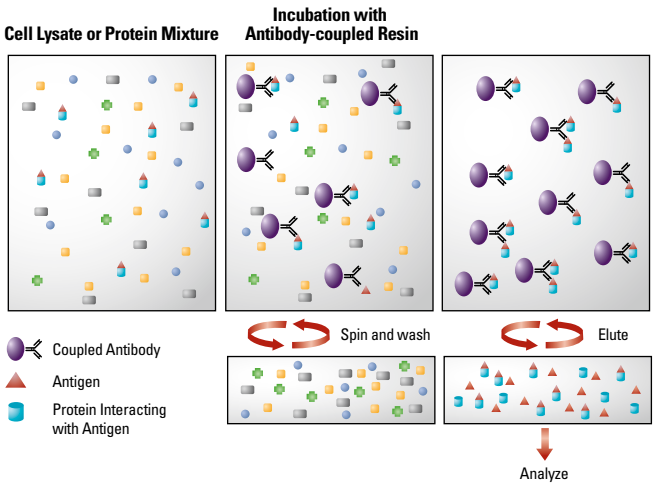


Figure 2. Summary of a traditional co-immunoprecipitation procedure.

Traditional Methods vs. Thermo Scientific Pierce Innovations for Co-IP

Problems with Traditional Co-IP Methods

The traditional co-IP protocol has certain deficiencies relating to the fundamental format of the assay, the antibody and associated chemistry. One of the most commonly encountered problems with the traditional IP and co-IP approach is interference from antibody bands in gel analysis. In those cases in which several proteins may be co-precipitated with the target, presence of the co-eluted antibody heavy and light chains (25 and 50 kDa bands in reducing SDS-polyacrylamide gel) in the preparation can obscure the results. The ideal situation would be to conduct the co-IP without contamination of the eluted antigen with antibody. With this potential interference eliminated, only the co-precipitated proteins will be present and detected on a gel. This and other shortcomings of the traditional protocol and our solutions are summarized in Table 1.

Table 1. Comparison of traditional Co-IP and Thermo Scientific Pierce Co-IP Products.

Traditional Co-IP Problems	Thermo Scientific Pierce Product Solutions
Batch processing of the precipitated complex in a single tube: results in inefficient washing of non-bound proteins from the support and in resin loss due to decanting wash buffer from tube via a pipette, which lowers protein yields	**Spin cup or spin tube processing:** dedicated IP and co-IP kits that contain spin-cup or spin tube devices that increase washing efficiency offer more effective elution of antigen and associated protein and eliminate resin loss, yielding significantly higher protein recoveries and more consistent results
Antibody fragment interference: co-elution of antibody fragments with antigen often results in bands interfering with detection of any co-precipitated proteins on SDS-PAGE	**Antibody immobilization:** chemistries designed to immobilize the antibody to the support, thereby allowing elution of only the target and any associated proteins in a co-IP complex **Eliminate detection of antibody used for immunoprecipitation:** contaminating antibody fragments become denatured during SDS-PAGE. In contrast, the primary antibody used during Western blotting is still native. Use CleanBlot HRP Detection Reagent (Product # 21230) to only detect native antibody and not contaminants.
Antibody sacrificed: as a consequence of harsh elution conditions, the target antibody is destroyed; antibody loss by way of the protocol can be costly	**Antibody re-used:** immobilization chemistry and mild elution conditions for the target and associated proteins allow the immobilized antibody to be re-equilibrated and recycled several times in the co-IP protocol

Approaches to Co-IP Free of Antibody Interference

Three approaches have been incorporated into several products targeted to IP and co-IP applications.

Activated Support for Antibody Immobilization — Direct Strategy

In this approach, an antibody is immobilized directly through its surface amine groups (contributed primarily by the side chain epsilon-amino group of lysine) to a high-capacity aldehyde-activated beaded agarose support (Thermo Scientific AminoLink Plus Coupling Resin). The support forms a Schiff's base with these available amines that is reduced to form stable secondary amine bonds during the immobilization process. The wide range of coupling conditions that can be used with this support make it ideal for maintaining biological binding activity critical to the successful execution of a co-IP experiment. When using the Direct Strategy, the antibody source should be free of carrier protein, which can also be immobilized. Products such as Thermo Scientific Melon Gel IgG Purification Kits (Product # 45206) can easily clean-up an antibody to remove carrier proteins. The direct immobilization of antibodies is not species dependent, which allows for the use on non-traditional antibody sources that don't bind globulin binding proteins (Protein A or Protein G). Thermo Scientific Pierce Co-Immunoprecipitation Kits (Product # 26149) and Thermo Scientific Pierce Direct IP Kit (Product # 26148) use this direct immobilization approach. For co-IP applications, the flexibility, simplicity and d urability of the direct method as an antibody-coupling strategy makes it the method of choice for delivering results free from antibody interference.

Antibody Orientation and Immobilization — Indirect Strategy

This strategy takes advantage of the binding characteristics of the traditional Protein A or Protein G agarose combined with chemical crosslinking to covalently link the antibody to the support. Protein A and Protein G bind IgG class antibodies through the Fc region that is characterized primarily by dimerized heavy chain modified by carbohydrate. Fc region binding naturally orients the antigen-binding domains of the antibody (Fab) away from the support, making them available for binding to their respective target antigen. The Indirect Strategy is compatible with antibody samples that contain carrier proteins, provided the carrier proteins are washed away prior to crosslinking. To ensure that the antibody remains on the support during the requisite antigen binding, wash and elution steps of the protocol, this bound and oriented antibody is chemically crosslinked to the Protein A or Protein G with the bifunctional reagent disuccinimidyl suberate (DSS). Thermo Scientific Pierce Crosslink IP Kit (Product # 26147) incorporates this strategy.

Use of the Streptavidin:Biotin Interaction

This direct coupling approach incorporates the binding association between streptavidin and biotin. Streptavidin immobilized to beaded agarose resin or coated in microplate wells provides an alternative IP or co-IP strategy for obtaining results free from antibody interference. Biotinylated antibody is bound very strongly to each matrix and is not eluted when mild conditions are used to release the target antigen. The IP/co-IP is conducted by incubating the sample with the biotinylated antibody-loaded matrix. Elution of the target antigen and any interacting proteins is performed free of antibody contamination. Thermo Scientific Pierce Streptavidin Agarose Resin (e.g., Product # 20347) and kit products that use this support, as well as the Thermo Scientific Pierce Streptavidin Coated Plate IP Kit (Product # 45360), provide high-capacity biotin-binding matrices suitable for IP and co-IP applications.

Optimization Parameters in IP and Co-IP

Classical Immune Complex Formation vs. Pre-Binding of Antibody

A change in protocol from the classical immune complex precipitation is necessary when using immobilized antibody in the co-IP method. In the traditional co-IP protocol, the immune complex (antigen:antibody) is formed in solution before "precipitating" it with the immobilized Protein A or Protein G matrix. When using immobilized antibody, the immune complex is formed directly on the antibody-coupled matrix by incubation of the antigen-containing sample with the matrix. Formation of the immune complex (the target antigen and any target-associated protein) and its precipitation occurs in one step.

In the immobilized format, the antibody is allowed to incubate with the lysate. The matrix is washed using a spin cup format and the bound protein eluted for analysis. The target antigen and co-IP complex is recovered free of antibody or antibody fragment contamination, and the antibody is retained in an active form on the support to be used in another co-IP cycle.

Our research indicates that pre-binding or -coupling the antibody to the support matrix consistently results in the capture of more target antigen, even in coated plate IP procedures that do not require it. This approach is recommended for the Thermo Scientific Pierce Coated Plate IP Kits that use 96-well microplates coated with streptavidin or Protein A/G (Products # 45360, 45350, respectively). Antibody is bound to the plate wells prior to the prescribed incubation with a lysate sample. Unbound protein is easily washed from the wells prior to the elution of the target and any co-precipitated proteins.

Evaluating a Co-IP-Captured Interaction

In their review of protein interactions, Phizicky and Fields (see References listed below) present a discussion of the issues to consider in validating a suspected interaction obtained by a co-IP experiment. Ultimately, the following question must be answered: Does the interaction detected by co-IP occur *in vivo*, and what significance does it have at the cellular level? A summary of the Phizicky and Fields approach to verification of co-IP data follows.

Confirm that the co-precipitated protein is obtained only by antibody against the target

Use monoclonal antibodies in the co-IP protocol. When only a polyclonal antibody is available, pre-treatment of the antibody with sample devoid of the primary target (bait protein) may be required to assure that the polyclonal antibody does not contain clones or contaminants that bind prey protein(s) directly. Pre-adsorption to extracts devoid of target or pre-purification of polyclonal IP antibodies against an affinity column containing pure target antigen safeguards against a false-positive co-IP.

Conclude that antibody against the target antigen does not itself recognize the co-precipitated protein(s)

Use independently derived antibodies that have demonstrated specificities against different epitopes on the target protein. Their use serves as verification that the target (bait)-directed antibodies have no affinity for the target-associated prey proteins recovered during the co-IP. Alternatively, an antibody against the co-precipitated protein can be used to co-IP the same complex.

Determine if the interaction is direct or indirect

Is the interaction mediated through a third-party protein that contacts both target and co-precipitated protein? Immunological and other more sophisticated methods such as mass spectrometry may be necessary to answer this question.

Determine that the interaction takes place in the cell and not as a consequence of cell lysis

Suggested approaches here involve co-localization studies and site-specific mutagenesis giving rise to mutants that perturb the binding process.

References

Adams, P.D., *et al.* (2002). Identification of associated proteins by co-immunoprecipitation, *In Protein-Protein Interactions – A Molecular Cloning Manual.* Golemis, E., Ed., Cold Spring Harbor Laboratory Press, pp 59-74.

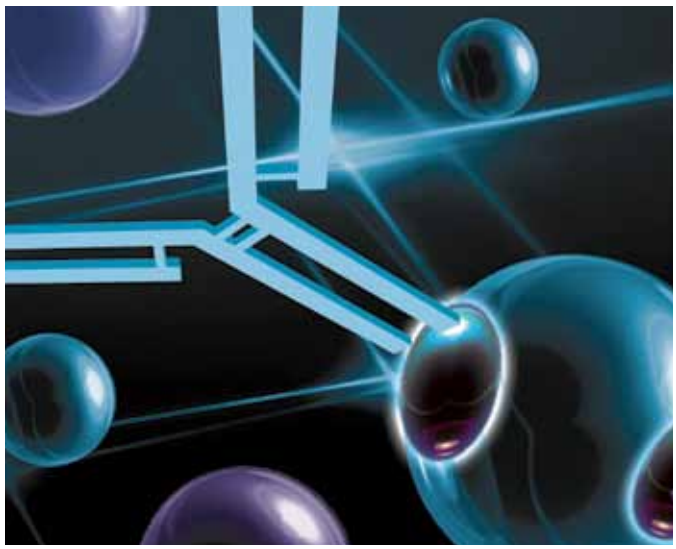
Liebler, D.C. (2002). Identifying protein-protein interactions and protein complexes. *In Introduction to Proteomics, Tools for the New Biology*, Humana Press, pp.151-165. (Product # 20061)

Phizicky, E.M. and Fields, S. (1995). Protein-protein interactions: methods for detection and analysis. *Microbiological Reviews* (Mar.), pp. 94-123.

IP and Co-IP Kits

The Thermo Scientific Pierce Classic and Crosslink IP Kits are ideal when using antibodies that bind to Protein A or Protein G. The Direct and Crosslink IP Kits are highly effective for eliminating co-elution of IgG heavy and light chain with the antigen, which interferes with downstream applications such as mass spectrometry analysis or protein sequencing.

Traditional co-IP methods result in detection of the antibody with the target proteins. Because the antibody heavy and light chains may co-migrate with one of the relevant bands, important results can be masked. The Pierce Co-IP Kit circumvents issues with co-migration of antibody chains with target proteins by retaining the antibody on the resin. The new kit is optimized for using smaller amounts of sample and offers a single lysis/wash buffer eliminating the need for a separate lysis reagent.



All four IP Kits Highlights:

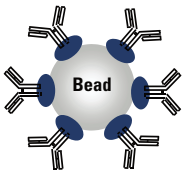
- Require minimal antibody (2-10µg)
- Are highly effective and efficient in capturing antigens
- Use optimized protocols and buffers for efficient IPs and antigen elution
- Use common lysis/binding/wash buffer
- Include spin columns and collection tubes that shorten the protocol by minimizing handling and mixing
- Use a new elution buffer that provides milder and less denaturing recovery of antibody:antigen complexes
- Are compatible with specialized downstream applications; e.g., mass spectrometry, enzyme assays and antibody production
- Are able to scale up as needed using our flexible protocol

Comparison of immunoprecipitation methods. The following table compares the key features of traditional "do-it-yourself" immunoprecipitation techniques to the Thermo Scientific Pierce IP Kits. Consideration of these features can help to determine which method is most appropriate for the available reagents and downstream application.

Feature	Traditional IP method	Pierce Classic IP Kit	Pierce Crosslink IP Kit	Pierce Direct IP Kit	Pierce Co-IP Kit
Uses high binding capacity resin	Variable[1]	Yes (Protein A/G Plus)	Yes (Protein A/G Plus)	Yes (AminoLink Plus)	Yes (AminoLink Plus)
Crosslinker mediated immobilization	No	No	Yes (DSS)	No	No
Requires purified antibody in amine-free and protein-free storage solution	No	No	No	Yes	Yes
Antibody is covalently attached to agarose resin	No	No	Yes	Yes	Yes
Antibody is oriented	Yes	Yes	Yes	No	No
Antibody elutes with antigen	Yes	Yes	No	No	No
Antigen recovery method	Boiling w/SDS (Low pH)	Low pH elution (Boiling w/SDS)	Low pH elution	Low pH elution	Low pH elution
Relative antigen recovery[2]	Variable	Highest	Medium	High	High
Immobilized antibody can be reused	No	No	Possible[3]	Possible[3]	Possible[3]
Co-purification of interacting proteins		Possible	Possible	Possible	Optimal

[1] Commercially available resins vary in binding capacity and performance for IP assays.
[2] Antigen yield depends on the activity of the antibody, specific binding conditions and the immobilization method used.
[3] It is possible to reuse the prepared antibody affinity resin if the antibody remains functional following low pH elution.

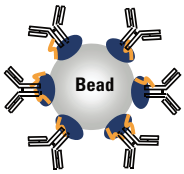
The Classic IP Kit:



Product # 26146

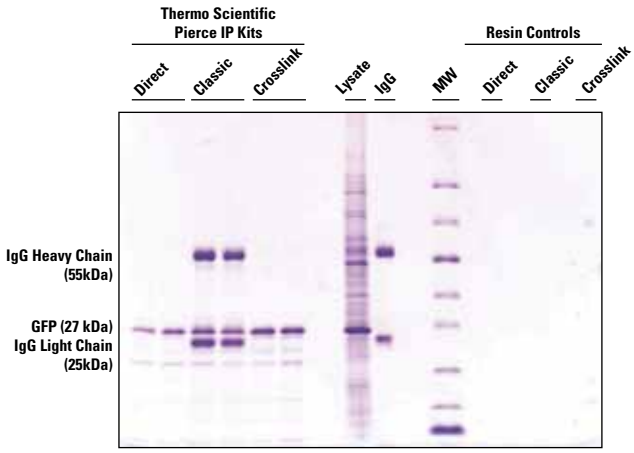
- High binding-capacity recombinant Protein A/G Plus Agarose Resin results in higher antigen yields
- Recombinant Protein A/G Plus Resin offers compatibility with a wider range of mammalian IgG species for IP reactions (e.g., mouse, rabbit, human and goat IgG subclasses)

The Crosslink IP Kit:



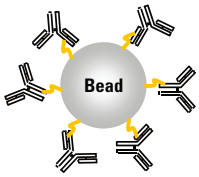
Product # 26147

- Able to purify target protein without contamination by the antibody in the eluate
- Improved crosslinking protocol optimized for maximum antibody functionality
- Bound immunoglobulins oriented for optimal antigen-binding sites are more accessible
- High-capacity Protein A/G Plus Resin allows for better purification and immobilization of antibodies
- Offers compatibility with a wider range of mammalian IgG species



Comparison of three different Thermo Scientific Pierce IP Kits. Immunoprecipitations were performed according to the product instructions using 10µg of affinity-purified goat anti-GFP antibody and the Pierce Direct, Classic and Crosslink IP Kits. The cell lysate was prepared using IP Lysis/Wash Buffer and pre-cleared using the Pierce Control Agarose Resin supplied in the kits. The immune complex was formed by incubating the antibody, resin and lysate overnight. The resin was washed with IP Lysis/Wash Buffer, 1X Conditioning Buffer and eluted with Elution Buffer. For analysis, 4-20% Tris-glycine gels were loaded with 20% of the eluted sample, 5% of the cell lysate load (Lysate) and 10% of the antibody load (IgG) and stained with Thermo Scientific Imperial Protein Stain (Product # 24615). For the resin controls, the immunoprecipitation was performed without adding the antibody.

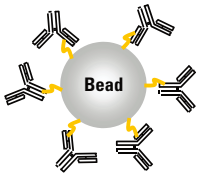
The Direct IP Kit:



Product # 26148

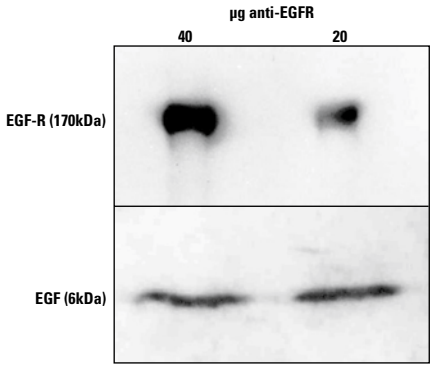
- Immobilize any antibodies independent of isotype or species
- Improved antibody coupling protocol
- Purify target protein without antibody contamination
- Activated resin for directly coupling antibodies to the support resin
- Eliminate antibody contamination in the eluate

The Co-IP Kit:



Product # 26149

- Minimal antibody requirements for co-IP reactions
- Shorter antibody coupling protocol (<2 hours)
- Compatible with any antibody species and subclass
- Requires a purified antibody in a solution free of amines and stabilizing proteins
- Optimized protocols and buffers for efficient co-IP and antigen elution
- Allows for selective purification of target protein
- Includes spin columns and collection tubes that shorten the protocol by minimizing handling and mixing
- Compatible with specialized downstream applications, e.g., mass spectrometry
- Allows for scale up



Co-immunoprecipitation of interacting proteins using the Thermo Scientific Pierce Co-IP Kit. Epidermal Growth Factor (EGF) was co-immunoprecipitated from 2,000µg of HeLa lysate with 40 and 20µg anti-Epidermal Growth Factor Receptor (EGFR) mouse monoclonal IgG_1 + IgG_{2a} (Thermo Scientific) using the Pierce Co-IP Kit. Co-immunoprecipitations were performed according to product instructions. Two identical Western blots were probed with anti-EGFR sheep polyclonal IgG (Millipore) or anti-EGF rabbit polyclonal IgG (Santa Cruz). The Pierce Co-IP Kit co-immunoprecipitated EGF with the EGF-R.

Thermo Scientific Pierce Classic IP Kit

A convenient kit for a new spin on traditional immunoprecipitation.

The Pierce Classic IP Kit provides all the necessary reagents, spin cups and collection tubes to perform successful immunoprecipitation (IP) experiments with ease. The kit uses high-capacity Protein A/G agarose affinity resin for efficient binding of most species and subclasses of IP antibodies. The included IgG elution buffer provides milder and less denaturing recovery of antibody:antigen complexes than the traditional method of boiling in reducing sample buffer for SDS-PAGE, facilitating a greater variety of methods for subsequent analysis. The microcentrifuge spin column format helps to ensure effective washing and separation of samples from the beaded agarose affinity resin.

Like traditional IP methods, the Pierce Classic IP Kit procedure involves formation of antibody:antigen complexes in a sample solution and then capture of that complex to an IgG-binding protein that is covalently bound to beaded agarose resin (Protein A/G Agarose). After washing to remove nonbound (presumably undesired) components of the sample, the antigen and antibody are recovered from the beaded resin with elution buffer supplied in the kit. The entire procedure is performed in a microcentrifuge spin column, allowing solutions to be fully separated from the agarose resin upon brief centrifugation.

Ordering Information

Product #	Description	Pkg. Size
26146	**Pierce Classic IP Kit**	Kit
	Sufficient reagents to perform 50 reactions.	
	Includes: Pierce Protein A/G Plus Agarose	0.55mL
	IP Lysis/Wash Buffer	2 x 50mL
	100X Conditioning Buffer	5mL
	20X Tris-Buffered Saline	25mL
	Elution Buffer	50mL
	5X Lane Marker Sample Buffer, Non-reducing	5mL
	Pierce Spin Columns – Screw Cap	50 each
	Microcentrifuge Collection Tubes	2mL, 100 each
	Microcentrifuge Sample Tubes	1.5mL, 50 each
	Pierce Control Agarose Resin	2mL

Thermo Scientific Pierce Crosslink IP Kit

Purify target protein complexes without antibody interference!

The Crosslink IP Kit extends the functionality of traditional immunoprecipitation (IP) methods by adding crosslinking technology and microcentrifuge spin column sample handling to the procedure. The primary benefits resulting from these features are the ability to purify target protein without contamination by the antibody and the ability to more effectively wash and separate samples from the beaded agarose resin.

The Pierce Crosslink IP Kit method involves capturing the IP antibody to Protein A/G Agarose resin and covalently immobilizing it to the support by crosslinking with disuccinmidyl suberate (DSS). The antibody resin is then incubated with the sample that contains the protein antigen of interest, allowing the antibody:antigen complex to form. After washing to remove nonbound (presumably undesired) components of the sample, the antigen is recovered by dissociation from the antibody with elution buffer supplied in the kit. The entire procedure is performed in a microcentrifuge spin cup, allowing solutions to be fully separated from the agarose resin upon brief centrifugation. Only antigen is eluted by the procedure, enabling it to be identified and further analyzed without interference from antibody fragments. Furthermore, the antibody resin often can be reused for additional rounds of immunoprecipitation.

Ordering Information

Product #	Description	Pkg. Size
26147	**Pierce Crosslink IP Kit**	Kit
	Sufficient reagents to perform 50 reactions.	
	Includes: Pierce Protein A/G Plus Agarose	0.55mL
	20X Coupling Buffer	25mL
	DSS Crosslinker, No-Weigh™ Format	8 x 2mg
	IP Lysis/Wash Buffer	2 x 50mL
	100X Conditioning Buffer	5mL
	20X Tris-Buffered Saline	25mL
	Elution Buffer	50mL
	Lane Marker Sample Buffer, Non-reducing, (5X)	5mL
	Pierce Spin Columns - Screw Cap	50 each
	Microcentrifuge Collection Tubes	2mL, 100 each
	Microcentrifuge Sample Tubes	1.5mL, 50 each
	Pierce Control Agarose Resin	2mL

Thermo Scientific Pierce Direct IP Kit

Immunoprecipitate using any antibody species or subclass! Eliminate antibody band contamination of IP products.

The Pierce Direct IP Kit represents a significant advancement in immunoprecipitation (IP) technology by replacing the use of immobilized Protein A or Protein G with a method for direct covalent attachment of antibodies to the beaded agarose resin.

The primary benefits resulting from this method are the opportunity to use any species or subclass of purified antibody (not just types that bind to Protein A or G) and the ability to purify target protein without contamination by the antibody. The method also makes it possible to immunoprecipitate antigens from serum samples without co-purifying non-target immunoglobulins. Finally, the kit uses microcentrifuge spin cups to effectively wash and separate samples from the beaded agarose resin.

Ordering Information

Product #	Description	Pkg. Size
26148	**Pierce Direct IP Kit** *Sufficient reagents to perform 50 reactions.*	Kit
	Includes: AminoLink Plus Coupling Resin	2mL
	20X Coupling Buffer	25mL
	Quenching Buffer	50mL
	Wash Solution	50mL
	5M Sodium Cyanoborohydride Solution	0.5mL
	IP Lysis/Wash Buffer	2 x 50mL
	100X Conditioning Buffer	5mL
	20X Tris-Buffered Saline	25mL
	Elution Buffer	50mL
	5X Lane Marker Sample Buffer	5mL
	Pierce Spin Columns – Screw Cap	50 each
	Microcentrifuge Collection Tubes	2mL, 100 each
	Microcentrifuge Sample Tubes	1.5mL, 50 each
	Pierce Control Agarose Resin	2mL

Thermo Scientific Pierce Co-Immunoprecipitation Kit

Perform co-immunoprecipitation experiments without antibody interference.

The Pierce Co-Immunoprecipitation (Co-IP) Kit enables isolation of native protein complexes from a lysate or other complex mixture by directly immobilizing purified antibodies onto an agarose support.

Co-IP is a common approach to study protein:protein interactions that uses an antibody to immunoprecipitate the antigen (bait protein) and co-immunoprecipitate any interacting proteins (prey proteins). Traditional co-IP methods that use Protein A or G result in co-elution of the antibody heavy and light chains that may co-migrate with relevant bands, masking important results. The Pierce Co-IP Kit resolves this issue by covalently coupling antibodies onto an amine-reactive resin. The kit includes optimized buffers for protein binding and recovery, reagents to perform control experiments and efficient spin columns and collection tubes, which shorten the protocol and minimize handling and mixing.

Ordering Information

Product #	Description	Pkg. Size
26149	**Pierce Co-Immunoprecipitation Kit*** *Sufficient reagents to perform 50 reactions.*	Kit
	Includes: AminoLink Plus Coupling Resin	2mL
	20X Coupling Buffer	25mL
	5M Sodium Cyanoborohydride Solution	0.5mL
	Quenching Buffer	50mL
	Wash Solution	60mL
	IP Lysis/Wash Buffer	2 x 50mL
	100X Conditioning Buffer	5mL
	Elution Buffer	50mL
	5X Lane Marker Sample Buffer, Non-reducing	5mL
	Pierce Control Agarose Resin	2mL
	20X Modified Dulbecco's PBS Buffer	25mL
	Pierce Spin Columns – Screw Cap	50 each
	Microcentrifuge Collection Tubes	100 each
	Microcentrifuge Sample Tubes	50 each

** The Thermo Scientific Pierce Co-IP Kit (Product # 23600) and the Pierce Mammalian Co-IP Kit (Product # 23605) were discontinued on December 31, 2009. The next-generation Pierce Co-IP Kit (Product # 26149) will replace these old kits. The new co-IP kit is optimized for using smaller amounts of sample and offers a common lysis/wash buffer eliminating the need for a separate lysis reagent (Thermo Scientific M-PER Mammalian Protein Extraction Reagent, Product # 78503) as was offered with the Mammalian Co-IP Kit.*

Thermo Scientific Pierce Coated Plate Immunoprecipitation Kits

Pre-coated 96-well plates are easier to use and faster than traditional microcentrifuge tube methods.

Thermo Scientific Pierce Coated Plate IP Kits enable rapid immunoprecipitation of multiple samples without the usual tedium of pipetting, centrifuging and separating beaded affinity resin in individual microcentrifuge tubes. Immunoprecipitation is accomplished using coated 96-well microplates rather than beaded agarose resin. The plate format allows fast processing of multiple samples. Select from Protein A/G-, Protein G- or streptavidin-coated plates.

Highlights:

- Ready-to-use, high-quality coated plates provide high capacity and consistency
- Plate format best suited for simultaneously processing multiple samples and their control conditions
- Faster, easier and more thorough washing than with traditional tube/resin IP methods
- Uses familiar and convenient ELISA tools (multichannel pipettors and plate washing); no tedious separation of supernatant from pelleted resin beads, and no tubes to open, close and centrifuge
- Coated plates are 96-well strip plates, convenient for experiments requiring only a partial plate
- Easy-to-follow instructions, including detailed explanation of appropriate controls
- Three kits available, suitable for most common antibody types (mouse, rabbit, human and goat IgG subclasses) or any biotinylated antibody or "bait" protein

Thermo Scientific Pierce Protein A/G Coated Plate IP Kit (Product # 45350) immunoprecipitation of CD71 (transferring receptor) from human serum using and a goat anti-CD71 polyclonal antibody. Eluted products for the experimental and control samples were mixed with nonreducing sample loading buffer, separated by SDS-PAGE, transferred to nitrocellulose membrane and detected by Western blotting with the IP antibody, Goat-anti-mouse-HRP conjugated secondary antibody (Product # 31432) and Thermo Scientific SuperSignal West Dura Chemiluminescent Substrate (Product # 34076).
Lane 1: Experiment (immunoprecipitation product)
Lane 2: Antibody-only control (no sample)
Lane 3: Human serum sample control (no antibody)
Lane 4: Plate control (no antibody or human sample)
Lane 5: Pure target protein (CD71) for size reference

Choosing between Protein A/G and Streptavidin Coated Plate Kits

Streptavidin is a protein that binds specifically and strongly to biotin; therefore, the Streptavidin Coated Plate IP Kit (Product # 45360) is appropriate for immunoprecipitation when using a biotin-labeled (biotinylated) antibody. This kit can be used to affinity-purify a binding partner to any antibody species or subclass or any other protein or molecule that is biotinylated. Because the streptavidin-biotin affinity interaction is so strong, the elution step generally will dissociate only the antigen (binding partner), not the biotinylated antibody or "bait" protein.

Protein A and Protein G are different proteins that bind to immunoglobulins (primarily IgG). Typically, Protein A is preferred for use with rabbit polyclonal antibodies, while Protein G is preferred for use with mouse antibodies (especially monoclonals of the IgG_1 subclass). Protein A/G is a recombinant of Protein A and Protein G that has the additive binding properties of both proteins.

Reference

Desai, S. and Hermanson, G. (1997). *Previews* **1(3)**, 2-7.

Ordering Information

Product #	Description	Pkg. Size
45350	**Pierce Protein A/G Coated Plate IP Kit** *Antibody binding capacity/well: 2.5µg. Sufficient capacity for downstream analysis of IP or co-IP proteins in-gel or by Western blot.* Includes: Protein A/G Coated 12 x 8-well strip plates Phosphate buffered saline Surfact-Amps® X-100 (10% Triton X-100) Elution buffer Neutralization buffer Uncoated 96-well strip plates (white), (for sample collection and neutralization) Plate sealers	Kit 2 plates 2 packs 6 x 10mL 50mL 7mL 2 ea. 18 sheets
45360	**Pierce Streptavidin Coated Plate IP Kit** *Antibody binding capacity/well: 5µg. Sufficient capacity for downstream analysis of IP or co-IP proteins in-gel or by Western blot.* Includes: High Binding Capacity Streptavidin Coated Plates Biotin blocking buffer Phosphate buffered saline Surfact-Amps X-100 (10% Triton X-100) Elution buffer Neutralization buffer Uncoated 96-well strip plates (white), (for sample collection and neutralization) Plate sealers	Kit 2 plates 30mL 2 packs 6 x 10mL 50mL 7mL 2 ea. 18 sheets

Thermo Scientific Pierce HA- or c-Myc Tag IP/ Co-Immunoprecipitation Kits

Need to perform IP or co-IP reactions with your HA- or c-Myc-tagged protein? Open box ... Read instructions ... Start performing an IP or co-IP. High-specificity immobilized antibodies make it easy.

No tags are more popular for mammalian system protein expression than HA or c-Myc. Although these tags are extremely popular, a kit that allows you to conveniently perform immunoprecipitation (IP) or co-immunoprecipitation (co-IP) reactions using these tags has not been available. The Pierce IP/Co-IP Kits include all necessary reagents, buffers and hardware that allow efficient purification of a tagged target protein (i.e., IP) or confirmation of potential interactions indicated by yeast two-hybrid results (i.e., co-IP).

These four kits, which are specifically for HA- and c-Myc-tagged proteins, allow you to easily perform an IP or co-IP experiment with minimal optimization. High-affinity, high-specificity antibodies immobilized onto an agarose matrix are at the heart of these new kits. In addition, the kits contain a full complement of buffers, eluents, a positive control and necessary hardware to efficiently perform the intended application.

Highlights:

IP and co-IP for HA- or c-Myc-tagged proteins directly out of the box.

- Demonstrated utility in the IP and co-IP application benefits the novice and expert. Our kits include all essential components to perform the assays. There's no need to formulate or validate raw materials.

Immobilized high-affinity antibodies with excellent specificity for the HA or c-Myc tag.

- The immobilized anti-HA and anti-c-Myc monoclonals precipitate the appropriately tagged protein specifically and in high yield, resulting in clean Western blot detection.
- Excellent results with as little as 2.5µg of anti-HA antibody and 1µg of anti-c-Myc antibody in the IP mode with the respective positive control lysate.
- Limits possibility of nonspecific binding to other proteins in the lysate.
- Eliminates contamination from antibody or antibody fragments after elution of the precipitated protein or the co-IP complex. This benefit is especially important when interpreting protein interaction results.

Simple, flexible and easy-to-follow protocols.

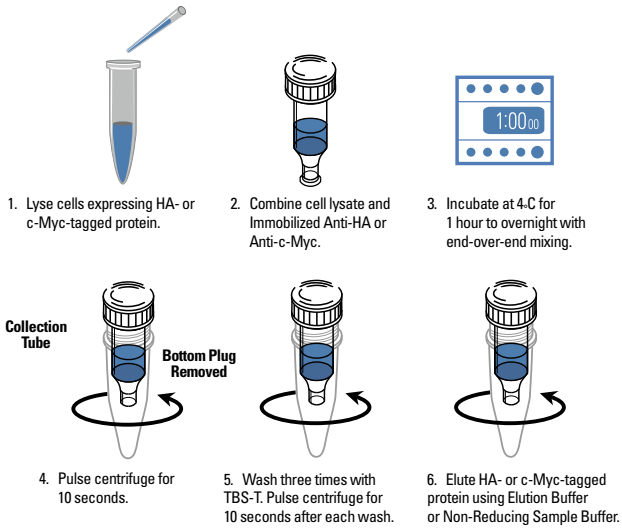
- Complete kit format offers optimum convenience in both the IP and co-IP modes.
- Eliminate Protein A or Protein G, reducing nonspecific binding and shortening the IP procedure.
- System demonstrates excellent flexibility with respect to the amount of antibody or amount of lysate used, enabling isolation of low-expression HA-/c-Myc-tagged targets.
- The use of Spin Columns accelerate the IP/co-IP process.

Spin Columns.

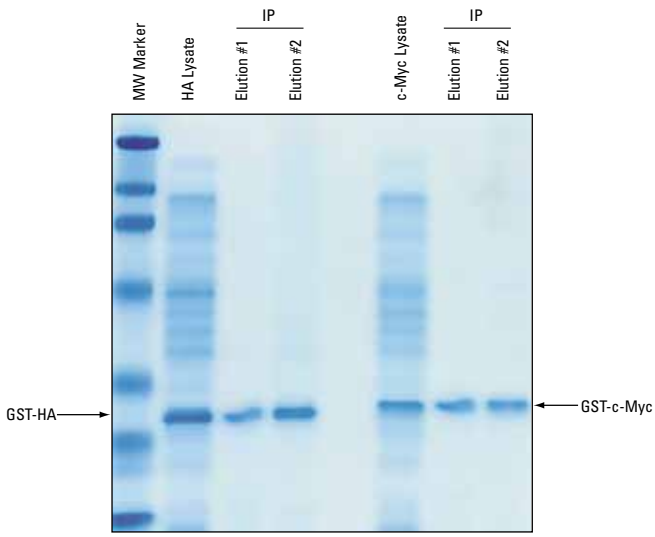
- Spin Columns are very convenient for small sample handling.
- Allow more efficient washing.
- Eliminate resin losses

HA- or c-Myc Tag IP/Co-IP Kit Descriptions

Each kit listed at right consists of two components: an Application Set and a Positive Control Lysate. The Application Set contains the immobilized support appropriate for the kit and all of the required buffers, eluents and hardware. The second component is a bacterial lysate containing an overexpressed GST with either HA or c-Myc as the C-terminal tag. The mammalian version of each kit contains Thermo Scientific M-PER Protein Extraction Reagent for use with mammalian cell-based IP or co-IP applications. The Application Sets and Positive Control Lysates can also be ordered separately.



Thermo Scientific Pierce HA- or c-Myc IP/Co-IP Kit Protocol summary.



Effectiveness of elution options in the IP of GST-HA and GST-c-Myc from bacterial lysates. IP results achieved with the Thermo Scientific Pierce HA and c-Myc IP/Co-IP Kits using the appropriate positive control lysate provided and suggested elution options for GST-HA and GST-cMyc, respectively. The elution components are supplied with each kit. Elution performed with **#1.** Elution Buffer or **#2.** 2X nonreducing sample buffer.

Ordering Information

Product #	Description	Pkg. Size
23610	**HA-Tag IP/Co-IP Kit** *Sufficient material to conduct 25 IP/co-IP assays using proteins expressed with an HA tag. Kit is supplied complete with an HA-tagged positive control lysate.* Includes: Immobilized Anti-HA (agarose resin) BupH Tris Buffered Saline Pack Elution Buffer, pH 2.8 Lane Marker Non-Reducing Sample Buffer (5X) Spin Columns Accessory Pack, 27 columns with pre-inserted frit and top and bottom caps Collection Tubes and Caps Accessory Pack, 100 graduated 2mL tubes and plug caps M-PER Mammalian Protein Extraction Reagent HA-Tagged Positive Control	Kit 150µL 1 pack 50mL 5mL 25mL 500µL
23615	**Mammalian HA-Tag IP/Co-IP Kit** *Sufficient material to conduct 25 IP/co-IP assays using proteins expressed with an HA tag. Kit is supplied complete with a mammalian cell lysis buffer and an HA-tagged positive control lysate.* Includes: Immobilized Anti-HA (agarose resin) BupH Tris Buffered Saline Pack Elution Buffer, pH 2.8 Lane Marker Non-Reducing Sample Buffer (5X) Spin Columns Accessory Pack, 27 columns with pre-inserted frit and top and bottom caps Collection Tubes and Caps Accessory Pack, 100 graduated 2mL tubes and plug caps M-PER Mammalian Protein Extraction Reagent HA-Tagged Positive Control	Kit 150µL 1 pack 50mL 5mL 25mL 500µL
23620	**c-Myc-Tag IP/Co-IP Kit** *Sufficient material to conduct 25 IP/co-IP assays using proteins expressed with a c-Myc tag. Kit is supplied complete with a c-Myc-tagged positive control lysate.* Includes: Immobilized Anti-c-Myc (agarose resin) BupH Tris Buffered Saline Pack Elution Buffer, pH 2.8 Lane Marker Non-Reducing Sample Buffer (5X) Spin Columns Accessory Pack, 27 columns with pre-inserted frit and top and bottom caps Collection Tubes and Caps Accessory Pack, 100 graduated 2mL tubes and plug caps c-Myc-Tagged Positive Control	Kit 250µL 1 pack 50mL 5mL 500µL
23625	**Mammalian c-Myc Tag IP/Co-IP Kit** *Sufficient material to conduct 25 IP/co-IP assays using proteins expressed with a c-Myc tag. Kit is supplied complete with a mammalian cell lysis buffer and a c-Myc-tagged positive control lysate.* Includes: Immobilized Anti-c-Myc (agarose resin) BupH Tris Buffered Saline Pack Elution Buffer, pH 2.8 Lane Marker Non-Reducing Sample Buffer (5X) Spin Columns Accessory Pack, 27 columns with pre-inserted frit and top and bottom caps Collection Tubes and Caps Accessory Pack, 100 graduated 2mL tubes and plug caps M-PER Mammalian Protein Extraction Reagent c-Myc-Tagged Positive Control	Kit 250µL 1 pack 50mL 5mL 25mL 500µL

Protein Interactions Technical Handbook

Our 72-page Protein Interaction Technical Handbook provides protocols and technical and product information to help maximize results for Protein Interaction studies. The handbook provides background, helpful hints and troubleshooting advice for immunoprecipitation and co-immunoprecipitation assays, pull-down assays, Far-Western blotting and crosslinking. The handbook also features an expanded section on method to study protein:nucleic acid interactions, including ChIP, EMSA and RNA EMSA. The handbook is an essential resource for any laboratory studying Protein Interactions. (# 1601618)

Protein Enrichment

Protein Enrichment

Protein enrichment encompasses numerous techniques to isolate subclasses of cellular proteins based on their unique biochemical activity, post-translational modification (PTM) or spatial localization in a cell. Protein enrichment is essential for studying low abundant proteins and for reducing the complexity of samples for proteomic analysis. Enrichment of specific proteins or protein complexes can most easily be accomplished using immunoaffinity techniques such as immunoprecipitation and co-immunoprecipitation. Although these antibody-based techniques are widely used, elution of immunoprecipitated proteins can sometimes result in low protein recovery or antibody contamination in samples.

Global protein enrichment strategies involve the selective isolation of distinct protein subclasses which share a common post-translational modification or cellular localization. Post-translational modifications such as phosphorylation and glycosylation can be enriched using affinity ligands such as ion-metal affinity chromatography (IMAC) or immobilized lectins, respectively. In addition, PTM-specific antibodies have been be used. Other techniques use metabolic or enzymatic incorporation of modified amino acids or PTMs to introduce unique protein chemistry which can be used for enrichment. Finally, proteins can also be enriched using various enzyme class specific compounds or cell-impermeable labeling reagents which selectively label cell surface proteins.

Phosphoprotein Enrichment Kits

Process cell and tissue samples in less time and with greater purity.

Phosphorylation is one of the most frequently occurring post-translational modifications in proteins. It is estimated that as many as 30% of all cellular proteins are transiently phosphorylated on serine, threonine and tyrosine residues.

Reversible protein phosphorylation regulates nearly all intracellular biological events, including signal transduction, protein-protein interactions, protein stability, protein localization, apoptosis and cell-cycle control. Deregulation of protein phosphorylation is a hallmark of numerous human diseases, including cancer and metabolic and immune disorders.

Detecting changes in protein phosphorylation can be a difficult task because of the transient labile state of the phosphate group. Furthermore, low phosphoprotein abundance and poorly developed phospho-specific antibodies contribute to difficulties in phosphoprotein detection. Recent advances in mass spectrometry technology in combination with phosphoprotein enrichment using immobilized metal affinity chromatography (IMAC) have resulted in greater resolution of the phosphoproteome.

The new Thermo Scientific Pierce Phosphoprotein Enrichment Kit efficiently enriches phosphorylated proteins derived from mammalian cells and tissues. The proprietary metal and buffer composition produces superior yields with negligible nonspecific binding.

Highlights:

- **Specific** – low contamination from nonspecific proteins
- **Fast** – easy-to-use spin format enriches of phosphorylated proteins in less than 2 hours
- **Superior yield** – high yield from complex biological samples, cell culture lysate and mouse tissue extract
- **Convenient format** – complete kit includes pre-dispensed spin columns, buffers, reagents and Thermo Scientific Pierce Protein Concentrators
- **Compatible** – works with downstream applications, including mass spectrometry, Western blotting and 2D-PAGE

Phospho-specific antibodies recognizing key regulatory proteins involved in growth factor signaling were used to monitor binding specificity of our Phosphoprotein Enrichment Kit (Figure 1). Specificity of the kit is further demonstrated by the absence of Cytochrome C (pI 9.6) and p15Ink4b (pI 5.5), two proteins not predicted to be phosphorylated, in the elution fraction and their emergence in the flow-through and wash fractions (Figure 1). Furthermore, dephosphorylation of HeLa cell extract *in vitro* resulted in diminished binding of PTEN, MAPK and GSK3β to the Pierce Phosphoprotein Enrichment Column as evidenced by their absence in the elution fraction. Conversely, all three proteins were present in the elution fraction from non-treated HeLa extract (Figure 2). Our Phosphoprotein Enrichment Kit provided superior and efficient phosphoprotein enrichment yields when compared to competitors' products (Table 1). It also effectively enriched phosphoproteins from homogenized mouse liver tissue (Figure 3).

Table 1. The Thermo Scientific Pierce Phosphoprotein Enrichment Kit provides higher phosphoprotein yields in less time than competitors' kits.

Kit	Yield (%)	Enrichment Time (Hours)
Thermo Scientific Pierce Phosphoprotein Enrichment Kit	15	1.5
Supplier Q Kit	4.4	4.5
Supplier I Kit	2.6*	3.5
Supplier C Kit	8	3
Supplier E Kit	Too dilute to determine	5

**Based on maximum 1mg load per manufacturer's protocol.*

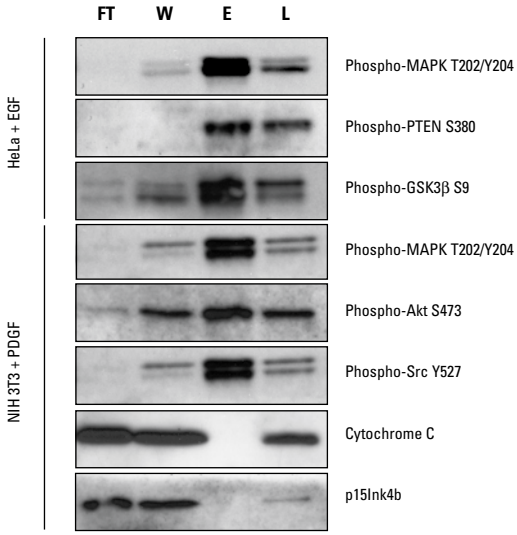


Figure 1. Highly pure phosphoprotein enrichment from complex biological samples. Serum-starved HeLa and NIH 3T3 cells were stimulated with EGF and PDGF, respectively. Cell lysate (2mg) was used for enrichment. Concentrated flow-through, wash and elution fractions were resolved by SDS-PAGE. Gel lanes were normalized by protein concentration (10µg/lane). Western blot analysis was performed using antibodies that detect site-specific phosphorylation events. Cytochrome C (pI 9.6) and p15Ink4b (pI 5.5) served as negative controls for nonspecific binding of non-phosphorylated proteins. **FT =** flow-through fraction, **W =** pooled wash fractions, **E =** pooled elution fractions and **L =** non-enriched total cell lysate.

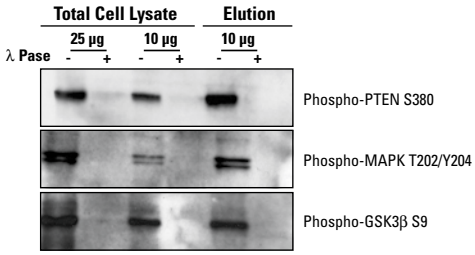


Figure 2. Highly specific phosphoprotein purification from lambda phosphatase-treated cells. Non-treated and lambda dephosphorylated HeLa cell extract (2mg) was loaded onto separate Thermo Scientific Pierce Phosphoprotein Enrichment Columns. Concentrated elution fractions were resolved by SDS-PAGE. Gel lanes were normalized by protein concentration (10µg/lane). To determine enrichment, 10µg and 25µg of non-treated or lambda phosphatase-treated total cell extract (non-enriched) was loaded onto each gel. Western blot analysis was performed using phospho-specific antibodies recognizing key proteins in the Ras-MAPK and PI3K-Akt signaling cascades.

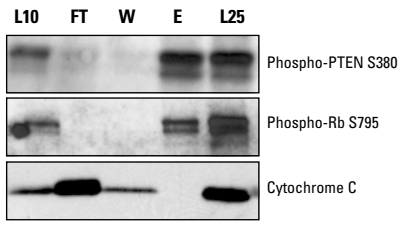


Figure 3. Efficient enrichment of phosphoproteins from mouse liver extract. Homogenized mouse liver extract (~2mg) was loaded onto a Thermo Scientific Pierce Phosphoprotein Enrichment Column. Concentrated flow-through, wash and elution fractions were resolved by SDS-PAGE. Gel lanes were normalized by protein concentration (10µg/lane). Western blot analysis was performed using antibodies that detect site-specific phosphorylation events. Cytochrome C (pI 9.6) served as a negative control for nonspecific binding. **L10 =** non-enriched total cell extract (10µg), **FT =** flow-through fraction, **W =** wash fraction, **E =** elution fraction and **L25 =** non-enriched total cell extract (25µg).

Ordering Information

Product #	Description	Pkg. Size
90003	**Pierce Phosphoprotein Enrichment Kit**	Kit
	Includes: Phosphoprotein Enrichment Column Resin Bed (1mL)	10 ea.
	Lysis/Binding/Wash Buffer	325mL
	Elution Buffer	60mL
	CHAPS	1g
	White Column Caps	10 caps
	Pierce Protein Concentrator 7mL/9K MWCO	10 Devices

Phosphoprotein Pull-Down with SH2 Domains

SH2 Domain Phosphotyrosine Capture Kits

SH2 domains provide an improved approach to selectively monitor receptor tyrosine kinase signaling, as well as the binding of downstream effector proteins. The interactions of SH2 domain-containing proteins represent a critical interface between extracellular stimulation of a membrane receptor and transmission of that signal to intracellular proteins.

Our kits include optimal levels of purified GST-fused SH2 domains of various signaling proteins integral to cell biology. Each validated kit includes optimized buffers and columns to perform protein pull-downs. Using SH2 domains eliminates the background associated with low-specificity antibodies and enables analysis of receptor targets to which antibodies are not available. Using quality-tested purified GST-SH2 domains also ensures uniform results without variability.

SH2 domains specifically bind phospho-tyrosine residues.

Each SH2 domain is specific for its natural target (Figure 4). *In vivo*, the phosphatase Shp2 is recruited to tyrosine 1009 of the platelet-derived growth factor receptor (PDGFR) only when it is phosphorylated. Also, PLCγ is recruited to tyrosine 1021 on PDGFR in response to growth factor signaling. In quiescent cells both tyrosine 1009 and 1021 are not phosphorylated and, therefore, both domains are unable to bind.

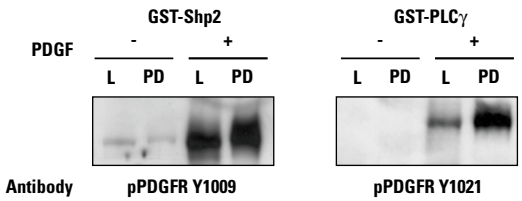
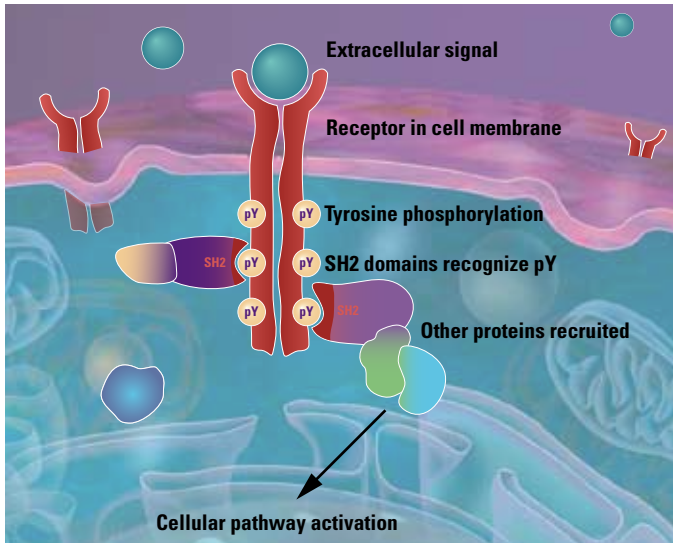


Figure 4. Site-specific interaction of GST-Shp2 and GST-PLCγ SH2 on the PDGF-receptor. NIH3T3 cells were rendered quiescent by serum withdrawal for 48 hours followed by stimulation with PDGF (50ng/mL, Cell Signaling Technology [CST]) for 15 minutes or no stimulation. Each cell lysate (500µg) was incubated overnight at 4°C with either 100µg GST-PLCγ1 or GST-Shp2. Protein complexes were captured on immobilized glutathione beads and resolved by SDS-PAGE. Western blot analysis was performed using phospho-specific antibodies that detect known protein-protein interaction sequences (phospho-PDGFR Y1009 and phospho-PDGFR Y1021, CST). Lanes: **L =** 25µg of total cell lysate and **PD =** SH2 domain pull-down.



SH2 domains provided a more efficient method of capturing site-specific phospho-tyrosine events as compared to antibody based co-immunoprecipitation.

Co-immunoprecipitation experiments are dependent on the quality and specificity of the antibody used. Antibodies against phosphoproteins are notoriously difficult to work with, yielding high background and low target protein recovery. SH2 domains provide better capture efficiency as compared to many pan- and site-specific phospho-antibodies because an SH2 domain is more selective by nature. Because of this improved specificity, SH2 domains can be used to pull-down phosphotyrosine containing proteins with improved results over traditional antibody-based methods. In Figure 5, PDGF-stimulated NIH3T3 cells were lysed and incubated with either a specific SH2 domain or a pan-antibody corresponding to the SH2 domain containing protein. Binding of each specific SH2 domain to the PDGF receptor was confirmed by Western blot using a pan-PDGFR antibody. In all cases we observed a higher capture efficiency using the SH2 domain approach as compared to the traditional antibody co-immunoprecipitation. In many cases, we were also able to detect differential binding of the SH2 domain in the presence of PDGF stimulation as compared to the unstimulated state. These differences were not apparent using the co-immunoprecipitation approach. We further tested the interaction specificity of four SH2 domains using phospho-antibodies which detect site-specific tyrosine phosphorylation events which mediate each SH2 domain interaction. We were limited to only four phospho-antibodies against specific tyrosine sites on the PDGF receptor because of commercial availability and/or antibody integrity. Using these site-specific antibodies we confirmed the selectivity and specificity of each SH2 domain tested and these interactions were much stronger as compared to the traditional antibody immunoprecipitation. Taken together these results clearly demonstrate that the SH2 domain pull-down approach represents a robust method to capture and monitor site specific tyrosine phosphorylation events which are critical for cell health.

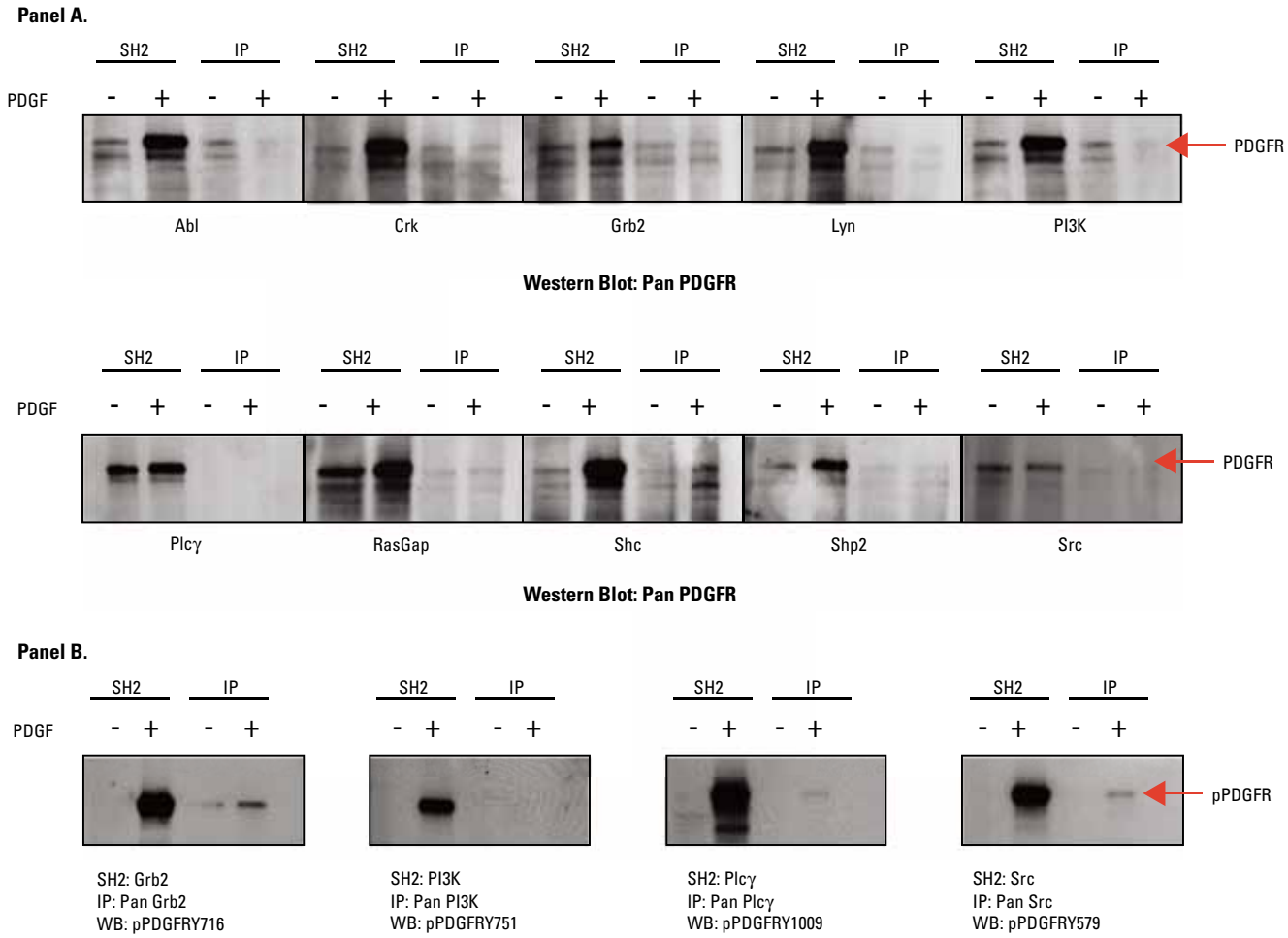


Figure 5. SH2 domains provide better enrichment of phosphorylated receptor tyrosine kinase (PDGFR) compared to antibody-based pull-down assays. Panel A. NIH3T3 cells were rendered quiescent by serum starvation for 24 hours with DMEM containing 0.1% FCS. Following starvation cells were either stimulated (100ng/mL PDGF for 20 minutes) or left unstimulated. Cells were then lysed in NP40 lysis buffer (50mm Tris pH 7.5, 150mm NaCl, 1% NP40, 5% glycerol) containing Thermo Scientific Halt Protease and Phosphatase Inhibitor Cocktail (Product # 78440) and protein concentrations determined by the Thermo Scientific Pierce 660nm Assay. SH2 and co-immunoprecipitations were performed as follows: SH2 domain pull-downs consisted of 100µg of SH2 domain (Abl, Crk, Grb2, Lyn, PI3K, Plc-gamma, RasGap, Shc, Shp2, Src) added to 250µg of stimulated or unstimulated lysate. Co-immunoprecipitations were performed by incubating 10µL of pan antibody (Abl, Crk, Grb2, Lyn, PI3K, Plc-gamma, RasGap, Shc, Shp2, Src) with 250µg of stimulated or non-stimulated lysate. Binding was performed for 16 hours at 4°C on a rotating platform. Following binding 100µL of a 50% slurry of immobilized glutathione was added to the SH2 domain samples and 100µL of a 50% slurry of Protein A/G was added to the co-immunoprecipitation samples. Samples were incubated for 1 hour at 4°C on a rocking platform followed by centrifugation at 1000 x *g* for 1 minute to collect complexes bound to the resin. Resin beds were then washed 3 times with 500µL of Thermo Scientific M-PER Wash Buffer. To elute bound proteins 25µL of 5X DTT loading dye was added to each sample and boiled for 5 minutes. Protein complexes were then resolved by SDS-PAGE on a 4-20% Tris-glycine gel. Proteins were transferred onto PVDF membrane for 16 hours at 4°C, 30V. Membranes were blocked in 7.5% BSA in TBST and Western blots performed using a 1:2000 dilution of mouse monoclonal anti-PDGFR-beta antibody (CST 3175). Detection was performed using Thermo Scientific SuperSignal Pico Chemilumescent Detection System. **Panel B.** Membranes containing the Grb2, Plcγ, PI3K, Src, and RasGap pull-downs were stripped with Thermo Scientific Restore Plus Buffer and reprobed with phospho-antibodies which detect site-specific tyrosine phosphorylation events on the PDGF receptor which mediate the SH2 domain interaction. These antibodies include pPDGFRY716, pPDGFRY751, pPDGFRY1009, pPDGFRY579 which correspond to SH2 domain docking sites for Grb2, PI3K, Plcγ, and Src respectively. Detection was performed using SuperSignal® West Pico Chemilumescent Detection System.

Phosphoprotein Pull-Down with SH2 Domains

Perform receptor phosphorylation time-course experiments.

With SH2 domain pull-downs, it is possible to monitor time-dependent binding of SH2 domains to their target. For example, PLCγ, Src and Shc bind rapidly to the PDGF receptor in response to PDGF stimulation (Figure 6). In nature, phosphorylation is rapid and transient, as evidenced by diminished binding at 10- and 20-minute post-stimulation.

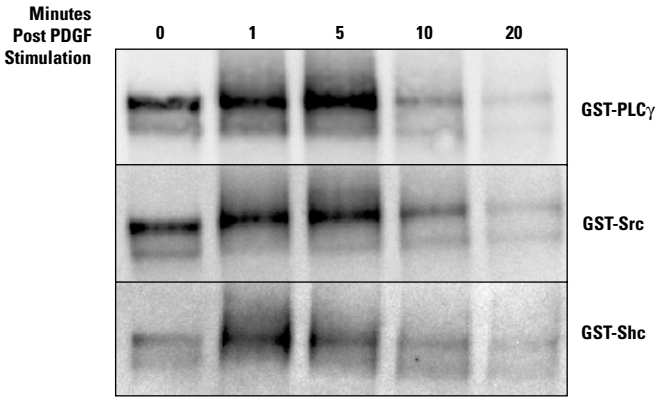


Figure 6. Time-specific binding of various SH2 domains to the PDGF receptor. NIH3T3 cells were rendered quiescent by serum withdrawal for 48 hours. Post-starvation cells were stimulated with PDGF (50ng/mL, CST) for the indicated times or untreated. SH2 domain pull-downs were performed with 100µg of each SH2 domain and 250µg of each cell lysate. Protein complexes were resolved by SDS-PAGE and analyzed by Western blot using a pan antibody recognizing PDGFR (CST).

Resolve specific tyrosine phosphorylation events.

SH2 domain affinity provides a level of selectivity not previously attainable with generic anti-phosphotyrosine antibodies. Use different SH2 domains to uncover the mechanism of how signals are transduced or to screen multiple SH2 domains against a particular target at one time. For example, different naturally occurring SH2 domains efficiently bind to the epidermal growth factor receptor (EGFR) in response to EGF stimulation (Figure 7). There were strong interactions of many of the domains in the presence of epidermal growth factor (EGF) and low-level binding in quiescent, serum-starved A431 cells. RasGap binding is independent of EGF stimulation, whereas Grb2 binding is highly upregulated (Figure 7).

Ordering Information

Product #	Description	Pkg. Size
87700	Grb2 SH2 Domain Phosphotyrosine Capture Kit *Sufficient reagents for six pull-down reactions (100µg/pull-down).* Includes: GST-Grb2 SH2 Domain (37 kDa) GST (negative control, 27 kDa) Immobilized Glutathione Resin (50% resin slurry) Spin Column (0.8mL) M-PER Mammalian Protein Extraction Reagent Lane Marker Reducing Sample Buffer (5X)	6 pull-downs 600µg at 1mg/mL 200µg at 1mg/mL 400µL 8 each 25mL 200µL
87701	Src SH2 Domain Phosphotyrosine Capture Kit	6 pull-downs
87702	Abl SH2 Domain Phosphotyrosine Capture Kit	6 pull-downs
87703	Crk SH2 Domain Phosphotyrosine Capture Kit	6 pull-downs
87704	Fyn SH2 Domain Phosphotyrosine Capture Kit	6 pull-downs
87705	Lck SH2 Domain Phosphotyrosine Capture Kit	6 pull-downs

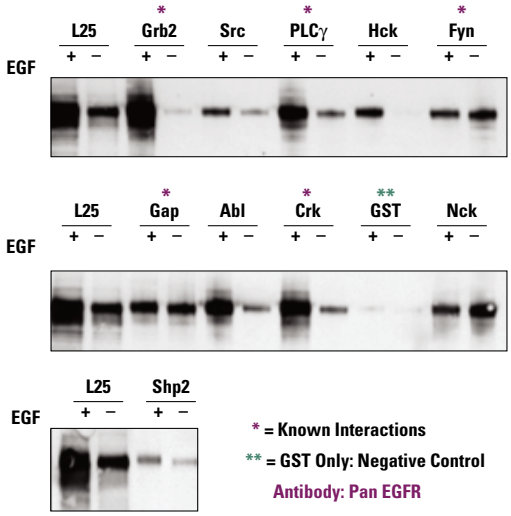


Figure 7. High efficiency binding of different naturally occurring SH2 domains to EGFR in response to EGF stimulation. A431 cells were rendered quiescent by serum withdrawal for 48 hours followed by stimulation with EGF (100ng/mL) for 15 minutes or left untreated. Each cell lysate (500µg) was incubated with 100µg of each GST-SH2 domain overnight at 4˚C. Protein complexes were captured on immobilized glutathione beads and resolved by SDS-PAGE. Western blot analysis was performed using a pan antibody that recognizes the EGF receptor. L25 = 25µg total lysate load.

Identify binding interactions with MS.

Pull-down samples isolated with SH2 domains are compatible with MS analysis for identifying protein interacting partners.

Improved specificity with SH2 domains and the elimination of antibody contamination typically seen in traditional immunoprecipitation experiments deliver pure protein for MS analysis. Two different SH2 domains were used to pull down interacting proteins from NIH3T3 cell lysates, which were then analyzed by MS. Many of the interacting proteins identified are specific to either Fgr or PLCγ.

Ordering Information

Product #	Description	Pkg. Size
87706	Nck SH2 Domain Phosphotyrosine Capture Kit	6 pull-downs
87707	Shc SH2 Domain Phosphotyrosine Capture Kit	6 pull-downs
87708	Ras-GAP SH2 Domain Phosphotyrosine Capture Kit	6 pull-downs
87709	Shp2 SH2 Domain Phosphotyrosine Capture Kit	6 pull-downs
87710	PLC SH2 Domain Phosphotyrosine Capture Kit	6 pull-downs
87711	Lyn SH2 Domain Phosphotyrosine Capture Kit	6 pull-downs
87712	Hck SH2 Domain Phosphotyrosine Capture Kit	6 pull-downs
87713	FGR SH2 Domain Phosphotyrosine Capture Kit	6 pull-downs
87714	Cbl SH2 Domain Phosphotyrosine Capture Kit	6 pull-downs
87715	Syk SH2 Domain Phosphotyrosine Capture Kit	6 pull-downs
87716	PI3K SH2 Domain Phosphotyrosine Capture Kit	6 pull-downs

Use of Titanium Dioxide for Phosphopeptide Enrichment

Titanium dioxide enrichment of phosphopeptide samples is an essential tool for comprehensive MS analysis of phosphopeptides. The unique phosphopeptide binding properties of TiO_2 resin results in different selectivity and preferential enrichment of multiply phosphorylated peptides compared to immobilized chelated metal ions (IMAC). TiO_2 resin typically enriches more phosphopeptides than IMAC resins, and TiO_2 preferentially enriches monophosphorylated peptides.

TiO_2 resin typically binds phosphopeptides with a higher affinity than IMAC and the preferential enrichment of singly phosphorylated peptides with TiO_2 is likely due to difficulty in eluting multiply phosphorylated peptides. To enrich a more comprehensive set of phosphopeptides, the flow-through and washes from IMAC resin can be acidified and applied to TiO_2 resins and the resulting eluates pooled for analysis. In addition, the number of phosphopeptides recovered can be significantly increased by pre-fractionation of samples with strong cation exchange chromatography to reduce sample complexity prior to enrichment with TiO_2 or IMAC processing.

Thermo Scientific Pierce TiO_2 Phosphopeptide Enrichment and Clean-up Kit

Selective enrichment and Clean-up of phosphopeptides in under 2 hours.

The Pierce TiO_2 Phosphopeptide Enrichment and Clean-up Kit enables fast, selective enrichment of phosphorylated peptides. The complete kit includes 24 titanium dioxide (TiO_2) spin tips and graphite spin columns with buffers to facilitate preparation of enriched and desalted phosphopeptides for analysis by mass spectrometry (MS).

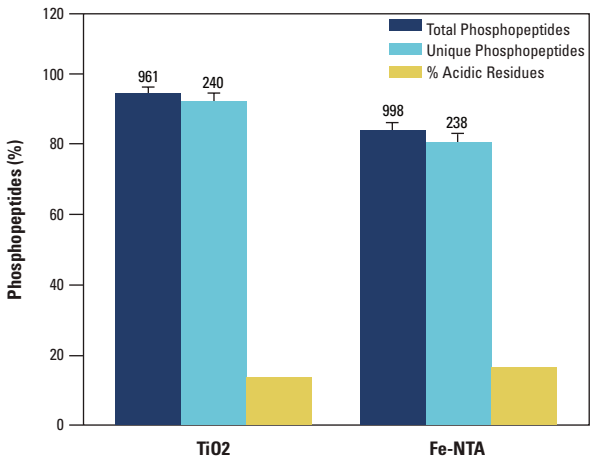
Highlights:

- **Convenient** – spin-column format of TiO_2 and graphite columns enable parallel processing and clean up of multiple samples in less than 2 hours
- **High capacity** – each column enriches up to 300µg of phosphopeptides
- **Complementary** – TiO_2 enriches a unique set of phosphopeptides that complements the Pierce Fe-NTA IMAC Phosphopeptide Enrichment Kit
- **High selectivity** – recover phosphopeptides with >90% selectivity

The Pierce TiO_2 Phosphopeptide Enrichment and Clean-up Kit protocol includes stringent washing conditions that increase the selectivity for phosphopeptides to >95%. Because some of the salts in these stringent washes may still be present in the eluted samples, the Pierce TiO_2 Phosphopeptide Enrichment and Clean-up Kit includes graphite spin columns to desalt and concentrate enriched phosphopeptides, resulting in more successful phosphopeptide analysis results. This kit is compatible with samples digested in solution or after in-gel digestion with trypsin or other MS grade proteases. Using the Pierce TiO_2 Phosphopeptide Enrichment and Clean-up Kit and the Pierce Fe-NTA IMAC Phosphopeptide Enrichment Kits will enable complementary sets of phosphopeptides to be removed from complex samples.

Number of Phosphates per PeptiOde							
Resin	1	2	3	4	5	6	**Total**
TiO_2	492	103	8	4	0	1	608
Fe-NTA IMAC	234	34	216	3	1	0	488
Overlap	155	0	1	0	0	0	156

Selective enrichment of singly and multiply phosphorylated phosphopeptides with Thermo Scientific Pierce TiO_2 and FE-NTA IMAC resins. Average phosphopeptide enrichment results from duplicate experiments showing the number of phosphopeptides containing one or more phosphate per peptide enriched using either resin. Peptide spectrum summary results were exported from Proteome Software Scaffold 3.0.



TiO_2 selectively enriches more phosphopeptides than Fe-NTA IMAC. The numbers refer to the total and unique number of phosphopeptides enriched by each method. The Y-axis is the percentage of phosphopeptides in the number of total and unique peptides, or the percentage of acidic residues in enriched unique phosphopeptides.

References:

Larsen M.R., *et al.* (2005). Highly selective enrichment of phosphorylated peptides from peptide mixtures using titanium dioxide microcolumns. *Mol Cell Proteomics.* **4(7)**:873-86.
Carrascal, M., *et al.* (2008). Phosphorylation Analysis of Primary Human T Lymphocytes Using Sequential IMAC and Titanium Oxide Enrichment. *J. Proteome Res.* **7(12)**:5167-5176.
Wilson-Grady, J.T., *et al.* (2008). Phosphoproteome Analysis of Fission Yeast. *J. Proteome Res.* **7(3)**:1088-1097.
Larsen, M.R., *et al.* (2004). Improved detection of hydrophilic phosphopeptides using graphite powder microcolumns and mass spectrometry: evidence for in vivo doubly phosphorylated dynamin I and dynamin III. *Mol. Cell Proteomics.* **3**:456-465.

Ordering Information

Product #	Description	Pkg. Size
88301	**Pierce TiO_2 Phosphopeptide Enrichment and Clean-up Kit** *Sufficient For: 24 phosphopeptide enrichment and cleanup reactions.* Kit Contents: TiO_2 Spin Tips, 24 tips Trifluoroacetic Acid, 1mL 90% Lactic Acid, 2mL Pyrrolidine, 200µL Centrifuge Column Adaptors, 24 adaptors Pierce Graphite Spin Columns, 24 columns	24-Rxn Kit

Phosphopeptide Enrichment

Thermo Scientific Pierce Magnetic Titanium Dioxide Phosphopeptide Enrichment Kit

TiO_2 magnetic particles for high throughput phosphopeptide isolation.

The Pierce Magnetic Titanium Dioxide Phosphopeptide Enrichment Kit is for isolating phosphopeptides from complex biological samples using titanium dioxide-coated magnetic beads. The TiO_2 ligand selectively binds peptides con-taining phosphorylated serine (Ser), tyrosine (Tyr) or threonine (Thr), enabling phosphopeptide enrichment from protease-digested samples. The isolated phosphopeptides are compatible for analysis downstream by mass spectrometry (Table 2).

The high-performance, iron oxide, superparamagnetic particles are validated and optimized for use with high-throughput magnetic platforms, such as the Thermo Scientific KingFisher 96 and KingFisher® Flex Instruments. The beads also enable premium performance for simple benchtop applications using an appropriate magnetic stand.

Highlights:

- **Complete MS-compatible Kits** – include ready-to-use binding, wash and elution buffers that are optimized for phosphopeptide enrichment and downstream analysis by MALDI and ESI mass spectrometry
- **Optimized for HTS** – procedure validated for processing 1 to 96 samples at a time; complete entire assay in about 15 minutes using a KingFisher Flex Instrument
- **Stable affinity ligand** – titanium dioxide is specially coated as a film on the magnetic particles
- **Selective** – affinity system is selective for phosphorylated Ser, Tyr and Thr; exhibits minimal non-specific binding to acidic residues
- **Sensitive** – affinity provides more than 1000 times greater sensitivity than traditional IMAC technologies; enables enrichment and MS-measurement of less than 100fmol of phosphoprotein

Table 2. Phosphopeptide enrichment improves MS-identification of phosphoproteins. Two milligrams of a tryptic digest prepared from periferal blood mononuclear cells (lymphocytes) with and without phosphopeptide enrichment were analyzed by MS. Enrichment was performed with the Thermo Scientific Pierce Titanium Dioxide Phosphopeptide Enrichment Kit using the Thermo Scientific KingFisher 96 Instrument. Samples were analyzed on a Thermo Scientific LTQ Orbitrap Mass Spectrometer.

	Enriched	Non-Enriched
Total number of proteins identified	185	247
Total number of phosphoproteins identified	160	1
Total number of peptides identified	2347	2457
Total number of phosphopeptides identified	2009	7
Total number of unique phosphopeptides identified	177	1
Relative enrichment for phosphopeptides (%)	86	0.3

Ordering Information

Product #	Description	Pkg. Size
88811	**Pierce Magnetic Titanium Dioxide Phosphopeptide Enrichment Kit**	Kit
	Includes: TiO_2 Magnetic Beads (20X)	1mL
	Binding Buffer	100mL
	Washing Buffer	25mL
	Elution Buffer	3mL
	Thermo-Fast 96 Robotic PCR Plate (0.2mL wells)	2 plates
88812	**Pierce Magnetic Titanium Dioxide Phosphopeptide Enrichment Kit, Trial Size**	Kit
	Includes: TiO_2 Magnetic Beads (20X)	0.25mL
	Binding Buffer	100mL
	Washing Buffer	25mL
	Elution Buffer	3mL
	Thermo-Fast 96 Robotic PCR Plate (0.2mL wells)	2 plates

Thermo Scientific Pierce Fe-NTA Phosphopeptide Enrichment Kit

New Fe-NTA format optimized for capture and recovery of phosphopeptides.

The new Pierce Fe-NTA Phosphopeptide Enrichment Kit enables fast and efficient enrichment of phosphorylated peptides. These spin columns are easy to use and require less than 1 hour to process protein digests or strong cation-exchange peptide fractions for analysis by mass spectrometry (MS).

Highlights:

- Convenient spin format for parallel processing of multiple samples
- High-binding capacity resin for enriching up to 150µg of phosphopeptides per column
- Excellent enrichment and recovery of phosphopeptides

Protein phosphorylation is essential to biological functions, including cell signaling, growth, differentiation, division and programmed cell death. Over 500 protein kinases catalyze phosphorylation of specific targets, primarily on serine, threonine, and tyrosine residues.

Mass spectrometry is increasingly being used to identify and quantify phosphorylation changes; however, phosphoprotein and phosphopeptide analysis by MS is limited by many factors, including digestion efficiency, low stoiochiometry, low abundance, hydrophilicity, poor ionization and poor fragmentation. As a result, phosphopeptide enrichment is essential to successful MS analysis. The new Pierce Fe-NTA Phosphopeptide Enrichment Kit is compatible with our lysis, reduction, alkylation, and digestion reagents and with Thermo Scientific Pierce Graphite Spin Columns to provide a complete workflow for phosphopeptide enrichment.

To assess phosphopeptide enrichment from lysates, cultured U2-OS cells arrested with nocodazole (100ng/mL, 25 hours) were lysed with 6M urea in 50mM Tris, pH 8.0 containing Thermo Scientific Halt Protease and Phosphatase Inhibitor Cocktail (Product # 78440). Protein concentration was determined with Thermo Scientific Pierce 660nm Protein Assay (Product # 22660). Proteins were reduced with Thermo Scientific Bond-Breaker TCEP Solution, Neutral pH (Product # 77720), alkylated with single-use iodoacetamide, (Product # 90034) digested overnight with MS-grade trypsin (Product # 90055), and desalted with Thermo Scientific HyperSep-C18 Cartridges (Product # 60108-305). An equivalent of 200µg of peptides were dried and dissolved in 5% acetic acid or Sigma Phos-Select™ Buffer. Phosphopeptides were enriched with Pierce Fe-NTA Phosphopeptide Enrichment Kit or Sigma Phos-Select Reagents and then desalted and concentrated with Pierce Graphite Spin Columns (Product # 88302) according to instructions.

Enriched phosphopeptide samples were analyzed by LC-MS/MS. A NanoLC™-2D HPLC (Eksigent) with a ProteoPep II C18 Column (75µm ID x 20cm, New Objective) was used to separate peptides using a 4-40% gradient of solvents (A: water, 0.1% formic acid; B: acetonitrile, 0.1% formic acid) at 250nL per minute for 60 minutes. Peptides were identified with a Thermo Scientific LTQ Orbitrap XL ETD Mass Spectrometer using a top four experiment consisting of high-resolution MS followed by acquisition of four MS/MS spectra using the CID mode of fragmentation. LC-MS/MS data were interpreted with Mascot 2.2 (Matrix Science) and Scaffold 2.6 (Proteome Software).

To achieve robust MS results, enrichment of phosphopeptide samples is essential because of low stoichiometry and abundance and poor ionization relative to nonphosphorylated peptides. We have developed an efficient means to enrich phosphopeptides from complex samples. The new Thermo Scientific Pierce Fe-NTA Spin Columns effectively capture, enrich, and recover phosphopeptides. These columns enrich a higher percentage of phosphopeptides than other resins and with an overall higher number of total and unique phosphopeptides (Figure 8 and Table 3).

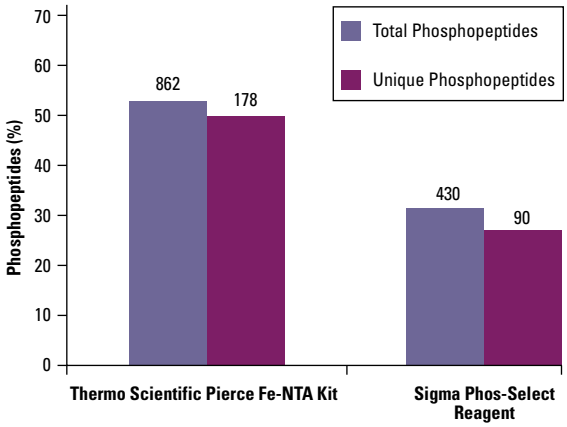


Figure 8. Our kit enriched a greater percentage of total and unique phosphopeptides from U2-OS cell lysate. Numbers refer to the total and unique number of phosphopeptides identified in each condition. A summary of results is listed in Table 3.

Table 3. Average phosphopeptide enrichment results from duplicate experiments.§

	Thermo Scientific Pierce Fe-NTA	Sigma Phos-Select
Total phosphopeptides	862	430
Total peptides	1,753	1,665
Total unique peptides	393	395
Total unique phosphopeptides	178	90
Total phosphopeptides (%)	53	31
Unique phosphopeptides (%)	50	27.5

§ Peptide summary results were exported from Scaffold and analyzed and summarized with Microsoft Excel® and Access®.

Multiple phosphorylated amino acids within a peptide contribute to the complexity of phosphopeptide analysis. Pierce Fe-NTA Spin Columns enrich peptides with three or more phosphorylated sites and significantly outperform other commercially available columns (Figure 9). Phosphopeptide enrichment greatly reduces sample complexity and enables effective identification and characterization of phosphorylated peptides by MS (Figure 10).

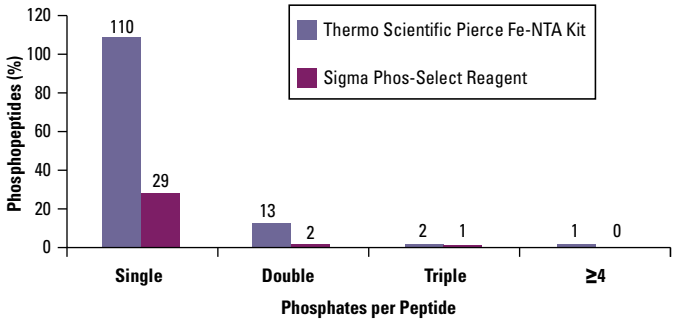


Figure 9. The Thermo Scientific Pierce Fe-NTA Phosphopeptide Enrichment Kit effectively captures phosphopeptides with multiple phosphates.

The Pierce Fe-NTA Phosphopeptide Enrichment Kit contains detailed instructions and all necessary components to load, wash and elute phosphopeptides within an hour. This kit is compatible with samples digested in solution or after in-gel digestion using the Thermo Scientific In-gel Tryptic Digestion Kit (Product # 89871).

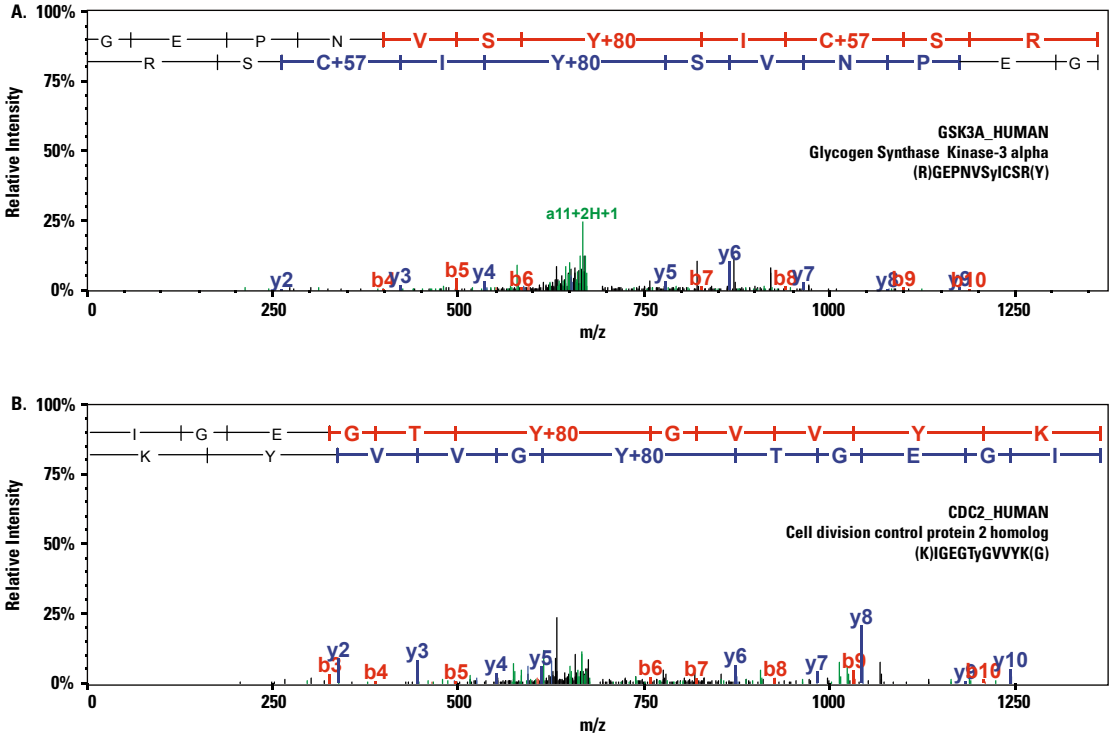


Figure 10. Example MS/MS spectra from enriched phosphopeptides. Lowercase letters indicate the position of tyrosine phosphorylation in enriched peptides. **Panel A:** Glycogen synthase-3 alpha. **Panel B:** cdc2/cyclin dependent kinase 1.

Ordering Information

Product #	Description	Pkg. Size
88300	Pierce Fe-NTA Phosphopeptide Enrichment Kit *Sufficient for 30 samples*	Kit

Related Products

88302	Pierce Graphite Spin Columns	30 columns

Cell Surface Protein Isolation Kit

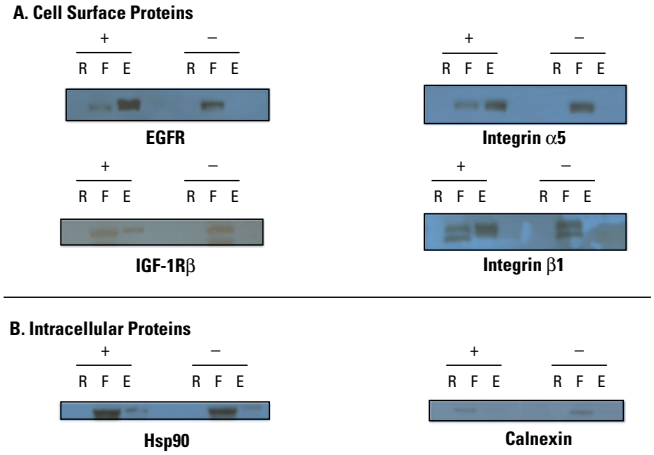
Convenient biotinylation and isolation of cell surface proteins for Western blot analysis.

The Thermo Scientific Pierce Cell Surface Protein Isolation Kit is a complete kit for the convenient biotinylation and isolation of mammalian cell surface proteins, specifically targeting cell surface proteins to the exclusion of intracellular proteins. The kit efficiently labels proteins with accessible lysine residues and sufficient extracellular exposure.

The isolation procedure uses a cell-impermeable, cleavable biotinylation reagent (Sulfo-NHS-SS-Biotin) to label surface proteins at exposed primary amines. Cells are then harvested and lysed, and the labeled surface proteins are affinity-purified using Thermo Scientific NeutrAvidin Agarose Resin. The isolated cell surface proteins contain a small, nonreactive tag of the originally labeled primary amines but are no longer biotinylated (biotin remains bound to the resin).

Highlights:

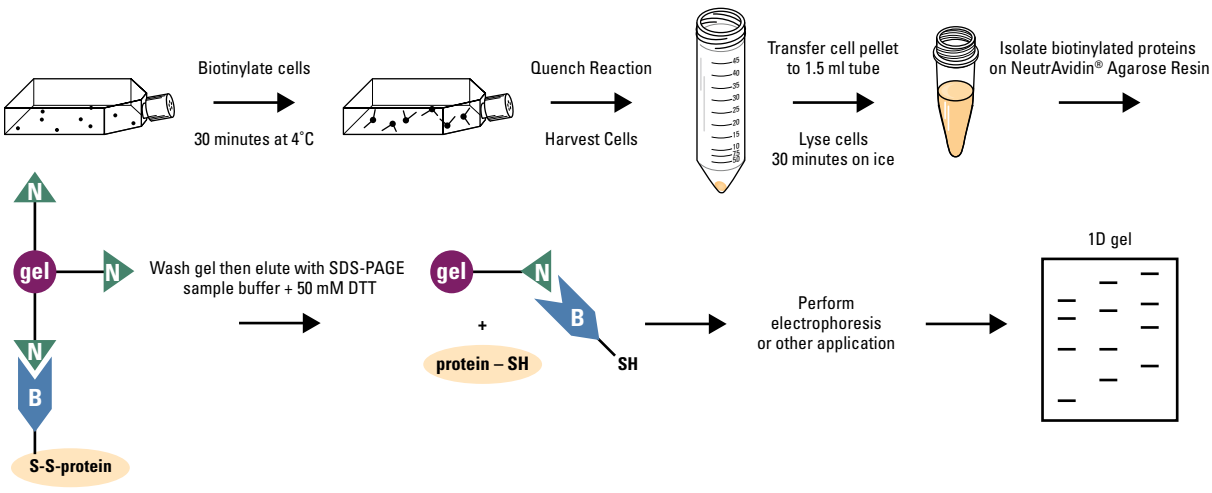
- **Isolates cell surface proteins** – reduces complexity of total cellular protein
- **Efficiently recovers labeled proteins** – cleavable biotin allows for nearly 100% recovery of isolated cell surface proteins
- **Convenience** – all reagents are provided in one kit, along with complete instructions for labeling, cell lysis and purification of cell surface membrane proteins
- **Western blotting applications** – proteins recovered in SDS-PAGE buffer are loaded directly onto polyacrylamide gels
- **Robust system** – protocol designed for diverse cell lines, including NIH 3T3, HeLa, C6 and A431



Protein isolation is specific to cell surface proteins. Panels are Western blot results for known cell surface proteins **(Panel A)** and intracellular proteins **(Panel B)** from HeLa cells tested with the Cell Surface Protein Isolation Kit. Plus symbol (+) denotes results for cells treated with the Sulfo-NHS-SS-Biotin reagent; minus symbol (-) denotes results for cells that were not treated with the biotin reagent but were otherwise carried through the kit procedure. Lanes are no-sample resin-control (R), flow-through (F) and eluted (E) fractions. Presence of target cell surface proteins in the plus-E and minus-F conditions indicate successful isolation with the kit. Presence of intracellular proteins in F condition of both plus and minus conditions indicates that the labeling and purification procedure is specific to cell surface proteins.

Ordering Information

Product #	Description	Pkg. Size
89881	**Cell Surface Protein Isolation Kit** *Sufficient reagents and accessories for eight experiments, each involving four T75 flasks of confluent cells.*	Kit
	Includes: EZ-Link® Sulfo-NHS-SS-Biotin	8 x 12mg vials
	Quenching Solution	16mL
	Lysis Buffer	4.5mL
	NeutrAvidin Agarose	2.25mL
	Wash Buffer	34mL
	Dithiothreitol (DTT)	8 x 7.7mg
	BupH Phosphate Buffered Saline	microtubes
	BupH Tris Buffered Saline	2 packs
	Spin Columns and Accessories	1 pack



Protocol summary for the Thermo Scientific Pierce Cell Surface Protein Isolation Kit.

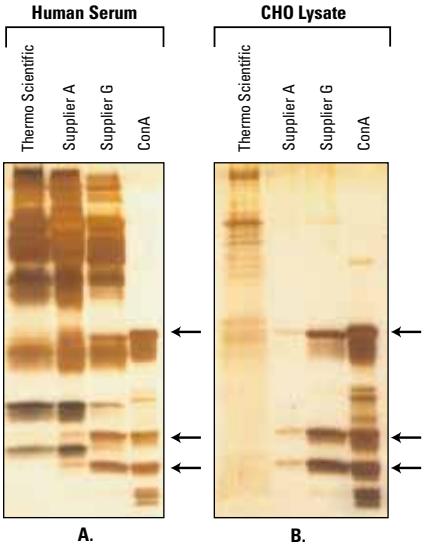
Glycoprotein Isolation Kits

Isolate glycoproteins from complex protein mixtures.

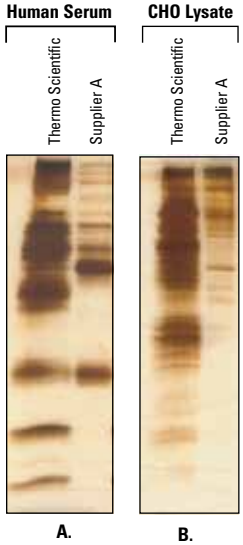
Two lectin-based Thermo Scientific Glycoprotein Isolation Kits, concanavalin A (ConA) and wheat germ agglutinin (WGA), allow isolation of glycoproteins from complex protein mixtures, including serum, tissue and cultured cell lysates, thus enabling downstream analysis. ConA lectin recognizes α-linked mannose and terminal glucose residues, while WGA lectin selectively binds to N-acetyl glucosamine (GlcNAc) groups and to sialic acid.

Highlights:

- **High recovery** – equivalent or greater glycoprotein recovery vs. competitor kits and lectin resins
- **Fast** – glycoprotein purification in less than one hour
- **Versatile** – isolate glycoproteins from various sample types; e.g., human serum and cell lysate
- **Robust** – lectin does not leach from resin when processing sample
- **Convenient** – complete kit contains lectin resins and spin columns with all necessary reagents
- **Compatible with Bradford-based protein assays** – dialysis or protein precipitation of recovered glycoproteins is not required before protein assay



Glycoprotein isolation from human serum and cell lysate: performance comparison of kits using ConA resin. Human serum and CHO lysate samples were processed with the Thermo Scientific Glycoprotein Isolation Kit, ConA and with other commercially available ConA resins. An equivalent amount of total protein was applied to each resin. Eluted glycoprotein fractions were compared with ConA Resin boiled in SDS-PAGE Buffer to release lectins. All fractions were normalized by volume and resolved on 8-16% polyacrylamide gels. Gels were silver-stained. **A.** Eluted glycoprotein fractions from applied human serum and **B.** eluted glycoprotein fractions from applied CHO lysate. Arrows identify protein bands that result from ConA leaching from the resin during the elution process.



Glycoprotein isolation from human serum and cell lysate: performance comparison of kits using WGA resin. Human serum and CHO lysate samples were processed with the Thermo Scientific Glycoprotein Isolation Kit, WGA and with other commercially available WGA resins. An equivalent amount of total protein was applied to each resin. Eluted glycoprotein fractions were normalized by volume and resolved on 8-16% polyacrylamide gels. **A.** Eluted glycoprotein fraction from applied human serum and **B.** eluted glycoprotein fraction from applied CHO lysate.

Ordering Information

Product #	Description	Pkg. Size
89804	**Glycoprotein Isolation Kit, ConA** *Sufficient reagents to isolate glycoproteins with strong affinity for ConA from 10 samples of up to 640µL (1-1.5mg total protein) each.* Includes: ConA Lectin Resin, 1.1mL resin supplied as a 50% slurry Binding/Wash Buffer, 5X Stock Solution Elution Buffer Column Accessory Pack, 10 Spin Columns with Caps and 20 Collection Tubes	Kit 6.5mL 5mL
89805	**Glycoprotein Isolation Kit, WGA** *Sufficient reagents to isolate glycoproteins with strong affinity for WGA from 10 samples of up to 640µL (1-1.5mg total protein) each.* Includes: WGA Lectin Resin, 1.1mL resin supplied as a 50% slurry Binding/Wash Buffer, 5X Stock Solution Elution Buffer Column Accessory Pack, 10 Spin Columns with Caps and 20 Collection Tubes	Kit 6.5mL 5mL

Ubiquitin Enrichment Kit

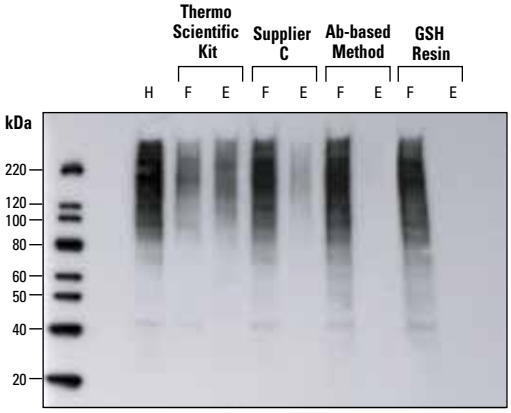
Recover ubiquitin modified protein in <45 minutes.

The ubiquitin proteasome pathway is the principal non-lysosomal pathway that controls the proteolysis of proteins. This pathway is significantly involved in a variety of cellular processes, including DNA repair, transcriptional regulation, signal transduction, cell metabolism and morphogenesis. Differences in total ubiquitination or the ubiquitination of specific proteins affect numerous pathological conditions, including malignancies, certain genetic diseases and neurodegenerative diseases.[1]

The Thermo Scientific Ubiquitin Enrichment Kit isolates polyubiquitin protein conjugates from cultured cells and tissue samples. The enriched fraction is analyzed to determine the amount of general ubiquitin conjugates present or to identify a specific protein by Western blotting. The assay protocol is fast, straightforward and allows isolation of polyubiquitinated proteins and the fractionation of monoubiquitinated species in the resin flow-through. The Ubiquitin Enrichment Kit outperforms other suppliers' kits and provides a clean and specific preparation of proteins when compared to a control resin.

Highlights:

- **Fast** – less than 45 minutes hands-on time
- **Complete** – includes all reagents needed for ubiquitin-modified protein enrichment from cultured cells and tissue samples, including spin columns and ubiquitin antibody
- **Flexible** – sample incubation from 2 hours to overnight allows assay to be completed in several hours or in less than 30 minutes after overnight incubation
- **Robust** – compatible with all Thermo Scientific Cell Lysis Solutions and standard RIPA formulations
- **Multiple-sample format** – easily processes 1-15 samples concurrently



The Thermo Scientific Ubiquitin Enrichment Kit recovers more ubiquitin-modified proteins than any other method. Epoxomicin-treated HeLa cell lysates (EHeLa, 150µg) were enriched. After elution, all samples were normalized to the initial load (EHeLa load). The flow-through and elution obtained using the Thermo Scientific Ubiquitin Enrichment Kit are shown first. The results using another supplier's enrichment kit are shown for comparison (Supplier C, manufacturer's instructions for this kit were followed). Additionally, the results obtained using an anti-ubiquitin monoclonal antibody-based enrichment scheme (antibody-based) and a negative control resin (GSH resin) are shown. The elution from each resin shows the amount of ubiquitin-modified protein that was captured using that method.

Reference

1. Ciechanover, A. (1998). The ubiquitin-proteasome pathway: on protein death and cell life. *EMBO J.* **17**(24), 7151-1760.

Ordering Information

Product #	Description	Pkg. Size
89899	**Ubiquitin Enrichment Kit** *Contains sufficient materials for enriching up to 15 lysate samples containing ~0.15mg total protein per sample.* **Pack 1** Polyubiquitin Positive Control (1,000X), 50µL, 2mg/mL Anti-ubiquitin Antibody, 50µL rabbit antiserum **Pack 2** Polyubiquitin Affinity Resin, 300µL, supplied as a 25% slurry Binding Capacity: ~1µg per 20µL of slurry BupH Tris Buffered Saline Pack, 1 ea., makes 500mL of 0.025M Tris, 0.15M NaCl; pH 7.2 Spin Columns and Accessories, 18 columns with top and bottom caps	Kit

Antibody Purification

Antibody Purification Overview

Antibodies specific for an antigen of interest are one of the most useful and important tools that biology researchers can possess. The production and use of specific antibodies as detection probes and purification ligands (i.e., immunotechnology) has revolutionized bioresearch and diagnostic technologies. Animals immunized with prepared antigens will produce specific antibodies against the antigen. When purified from serum or hybridoma cell lines that are prepared from tissue of the immunized animal, the antibody can be used directly (or after labeling with enzyme or fluorescent tags) to probe the specific antigen in Western blotting, ELISA or a variety of other applications. Antibodies are most commonly purified by one of two affinity purification methods: general immunoglobulin purification (pages 58-65) or specific antibody purification (see pages 26-35). See also Table 1.

General Purification of Immunoglobulins

Because antibodies have predictable structure, including relatively invariant domains, it has been possible to identify certain protein ligands that are capable of binding generally to antibodies, regardless of the antibody's specificity to antigen. Protein A, Protein G and Protein L are three bacterial proteins whose antibody-binding properties have been well characterized. These proteins have been produced recombinantly and used routinely for affinity purification of key antibody types from a variety of species. A genetically engineered recombinant form of Protein A and G, called Protein A/G, is also available. These antibody-binding proteins are available immobilized to beaded agarose resin, Thermo Scientific UltraLink Biosupport and coated onto microplates.

Proteins A, G, A/G and L bind to antibodies at sites other than the antigen-binding domain. Therefore, these proteins can be used in purification schemes such as immunoprecipitation.

Proteins A, G, A/G and L have unique properties, which make each one suitable for different types of antibody targets (e.g., antibody subclass or animal species). It is important to realize that use of Protein A, G or L results in purification of general immunoglobulin from a crude sample. Depending on the sample source, antigen-specific antibody may account for only a small portion of the total immunoglobulin in the sample. For example, generally only 2-5% of total IgG in mouse serum is specific for the antigen used to immunize the animal.

Table 1. Antibody Purification Methods.

	Purification Type	Description	Available Thermo Scientific Support
General	Negative selection	Removal of all non-immunoglobulin proteins from a serum sample	Melon Gel
	IgG enrichment	Immobilized globulin binding proteins to selectively remove IgG from a serum sample	Immobilized Protein A
			Immobilized Protein G
			Immobilized Protein A/G
			Immobilized Protein L
	IgG enrichment	Thiophilic adsorption	Thiophilic Adsorbent
	IgM enrichment	Use Mannan binding protein to selectively isolate IgM	Immobilized Mannan Binding Protein
	IgA enrichment	Use Jacalin, a D-galactose binding lectin, to selectively isolate IgA	Immobilized Jacalin
	IgY enrichment	Delipidation and precipitation from egg yolks	IgY Precipitation Reagent
Specific (see page 26)	Affinity purification	Create an affinity column with the antigen used to immunize the animal.	NHS-ester Activated Agarose - for immobilizing antigens via primary amines
			AminoLink Plus Agarose - for immobilizing antigens via primary amines
			SulfoLink Resin - for immobilizing antigens via reduced sulfhydryls
			CarboxyLink Resin - for immobilizing antigens via carboxylic acids

Immobilized Protein L, Protein A, Protein G and Protein A/G

We offer these popular antibody-binding proteins immobilized on several different resins, beads and plates for use in immunoaffinity purification techniques. All four proteins (A, G, A/G and L) are available as purified recombinants immobilized to crosslinked 6% beaded agarose. This is the traditional format historically used for small-scale column purification and immunoprecipitation methods. Our agarose resins differ from those typically offered by other suppliers in that our immobilization method is more stable and results in less nonspecific binding. We also offer "Plus" versions of the Protein A, G, A/G and L agarose resins, which contain twice the amount of protein per milliliter of resin and provide for nearly twice the antibody binding capacity.

Protein A, G, A/G and L are also available immobilized to UltraLink® Biosupport, an extremely durable, polyacrylamide-based resin with very low nonspecific binding characteristics. The UltraLink Format is a perfect support for working with large volume samples in large-scale purification methods requiring fast flow and high pressure.

The interaction between the various proteins and IgG is not equivalent for all species or all antibody subclasses. The tables on the following page will help you decide which affinity protein is best for your application (Tables 2 and 3).

Table 2. Characteristics of immunoglobulin-binding proteins.

	Recombinant Protein L	Native Protein A	Recombinant Protein A	Recombinant Protein G	Recombinant Protein A/G
Production Source	*E. coli*	*S. aureus*	*E. coli*	*E. coli*	*E. coli*
Molecular Weight	35,800	46,700	44,600	21,600	50,460
Number of Binding Sites for IgG	4	4	4	2	6
Albumin-Binding Site	No	No	No	No	No
Optimal Binding pH	7.5	8.2	8.2	5	5-8.2
Binds to	V_LK	F_c	F_c	F_c	F_c

Table 3. Binding characteristics of immunoglobulin-binding proteins and Thermo Scientific Thiophilic Adsorbent.*

	Protein A	Protein G	Protein A/G	Protein L†	Thiophilic Adsorbent
Human IgG	s	s	s	s	m
Mouse IgG	s	s	s	s	s
Rabbit IgG	s	s	s	w	m
Goat IgG	w	s	s	nb	s
Rat IgG	w	m	m	s	s
Sheep IgG	w	s	s	nb	s
Cow IgG	w	s	s	nb	s
Guinea Pig IgG	s	w	s	–	s
Hamster IgG	m	m	m	s	–
Pig IgG	s	w	s	s	s
Horse IgG	w	s	s	–	s
Donkey IgG	m	s	s	–	–
Dog IgG	s	w	s	–	s
Cat IgG	s	w	s	–	s
Monkey IgG (Rhesus)	s	s	s	–	s
Chicken IgY	nb	nb	nb	nb	m
Human IgM	w	nb	w	s	m
Human IgE	m	nb	m	s	–
Human IgD	nb	nb	nb	s	–
Human IgA	w	nb	w	s	m
Human IgA_1	w	nb	w	s	m
Human IgA_2	w	nb	w	s	m
Human IgG_1	s	s	s	s	m
Human IgG_2	s	s	s	s	m
Human IgG_3	w	s	s	s	m
Human IgG_4	s	s	s	s	m
Human Fab	w	w	w	s	m
Human ScFv	w	nb	w	s	m
Mouse IgG_1	w	m	m	s	s
Mouse IgG_{2a}	s	s	s	s	s
Mouse IgG_{2b}	s	s	s	s	s
Mouse IgG_3	s	s	s	s	s
Rat IgG_1	w	m	m	s	s
Rat IgG_{2a}	nb	s	s	s	s
Rat IgG_{2b}	nb	w	w	s	s
Rat IgG_{2c}	s	s	s	s	s
Cow IgG_1	w	s	s	nb	s
Cow IgG_2	s	s	s	nb	s
Sheep IgG_1	w	s	s	nb	s
Sheep IgG_2	s	s	s	nb	s
Goat IgG_1	w	s	s	nb	s
Goat IgG_2	s	s	s	nb	s
Horse IgG(ab)	w	nb	w	–	s
Horse IgG(c)	w	nb	w	–	s
Horse IgG(T)	nb	s	s	–	s
Mouse IgM	nb	nb	nb	s	m

w = weak binding, m = medium binding, s = strong binding, nb = no binding, – means information not available

** Data represent a summary of binding properties reported in the literature. Inevitably some discrepancies exist among reported values as a result of differences in binding buffer conditions and form of the proteins used.*

† Binding will occur only if the appropriate kappa light chains are present. Antibodies lambda light chains will not bind, regardless of their class and subclass.

Protein A

Protein A Beads – Quick Reference

Protein A	Native protein purified from *Staphylococcus aureus* (46.7kDa; four IgG-binding sites)
Specificity (Table 3)	Best for polyclonal IgG from rabbit, pig, dog, cat serum; poor for Mouse IgG1, human IgG3, rat, goat, cow
Supports Offered	Crosslinked 6% beaded agarose UltraLink Biosupport Trisacryl GF-2000 Resin Magnetic particles (1-4µm)
Package Formats	Resin slurries (3 sizes) Chromatography Cartridges (2 sizes) Packed Spin Columns (3 sizes) NAb™ IgG Purification Kits (2 sizes)
Storage	4°C, do not freeze
Capacity	Protein A: 12-19mg human IgG/mL resin Protein A Plus: >35mg human IgG/mL resin; 16-17mg mouse IgG/mL resin Protein A Trisacryl Resin: >15mg human IgG/mL resin Protein A UltraLink Resin: >16mg of human IgG/mL resin Protein A Plus UltraLink Resin: >30mg human IgG/mL resin Protein A MagnaBind Beads: >0.2mg rabbit IgG/mL beads Protein A Coated Plates: ~4pmol rabbit IgG/well Recombinant Protein A: ≥12mg human IgG/mL resin using the IgG Buffer System

Ordering Information

Product #	Description	Pkg. Size
20333	Protein A Agarose	5mL
20334	Protein A Agarose	25mL
20356	Protein A Columns	5 x 1mL
44667	Protein A IgG Purification Kit	Kit
20338	Protein A Trisacryl Resin	5mL
53139	Protein A UltraLink Resin	5mL
22810	Protein A Plus Agarose	1mL
22811	Protein A Plus Agarose	5mL
22812	Protein A Plus Agarose	25mL
89924	Chromatography Cartridges, Protein A	2 x 1mL
89925	Chromatography Cartridge, Protein A	1 x 5mL
89952	NAb Protein A Plus Spin Columns	10 x 0.2mL
89956	NAb Protein A Plus Spin Columns	5 x 1mL
89960	NAb Protein A Plus Spin Column	1 x 5mL
89948	NAb Protein A Plus Spin Purification Kit	Kit
89978	NAb Protein A Plus Spin Purification Kit	Kit
53142	Protein A UltraLink Plus Resin	5mL
45202	Protein A Spin Plate	1 plate
21348	MagnaBind Protein A Beads	5mL
15130	Protein A, Clear 96-Well Plates	5 plates
15132	Protein A, Clear 8-Well Strip Plates	5 plates
15154	Protein A, White 96-Well Plates	5 plates
15155	Protein A, Black 96-Well Plates	5 plates
20365	Recombinant Protein A Agarose	5mL
20366	Recombinant Protein A Agarose	25mL

Protein G

Protein G Beads – Quick Reference

Protein G	Recombinant protein expressed in *E. coli* (21.6kDa; two IgG binding sites)
Specificity (Table 3)	Best for IgG from mouse, human, cow, goat and sheep; poor for guinea pig, pig, dog and cat
Supports Offered	Crosslinked 6% beaded agarose UltraLink Biosupport Magnetic particles (1-4µm)
Package Formats	Resin slurries (3 sizes) Chromatography Cartridges (2 sizes) Packed Spin Columns (3 sizes) Nab IgG Purification Kits (2 sizes)
Storage	4°C, do not freeze
Capacity	Protein G: 11-15mg human IgG/mL resin Protein G UltraLink Resin: >20mg of human IgG/mL resin Protein G Plus: >20mg human IgG/mL resin Protein G UltraLink Resin: >20mg of human IgG/mL resin Protein G Plus UltraLink Resin: >25mg human IgG/mL resin MagnaBind Protein G Beads: >0.2mg rabbit IgG/mL beads Protein G Coated Plates: ~2pmol rabbit IgG/well Protein G Plus: >20mg human IgG/mL resin Protein G Plus UltraLink Resin: >25mg human IgG/mL resin

Ordering Information

Product #	Description	Pkg. Size
20398	Protein G Agarose	2mL
20399	Protein G Agarose	10mL
20397	Protein G Agarose	25mL
89926	Pierce Chromatography Cartridges, Protein G	2 x 1mL
89927	Pierce Chromatography Cartridge, Protein G	1 x 5mL
89953	NAb Protein G Spin Columns	10 x 0.2mL
89957	NAb Protein G Spin Columns	5 x 1mL
89961	NAb Protein G Spin Columns	1 x 5mL
89949	NAb Protein G Spin Purification Kit	Kit
89979	NAb Protein G Spin Purification Kit	Kit
21193	Pierce Recombinant Protein G	5mg
53125	Protein G UltraLink Resin	2mL
53126	Protein G UltraLink Resin	10mL
53127	Protein G UltraLink Columns	2 x 2mL
45204	Protein G Spin Plate	1 plate
22851	Protein G Plus Agarose	2mL
22852	Protein G Plus Agarose	10mL
53128	Protein G Plus UltraLink Resin	2mL
21349	MagnaBind Protein G Beads	5mL
15131	Protein G, Clear 96-Well Plates	5 plates
15133	Protein G, Clear 8-Well Strip Plates	5 plates
15156	Protein G, White 96-Well Plates	5 plates
15157	Protein G, Black 96-Well Plates	5 plates

Protein A/G

Protein A/G Beads – Quick Reference	
Protein A/G	Recombinant protein expressed in *E. coli* (50.5kDa; six IgG-binding sites)
Specificity (Table 3)	Best for polyclonal IgG from many species; poor for individual subclasses that have highest affinity for Protein A or Protein G.
Supports Offered	Crosslinked 6% beaded agarose UltraLink Biosupport
Package Formats	Resin slurries (3 sizes) Chromatography Cartridges (2 sizes) Packed Spin Columns (3 sizes) NAb IgG Purification Kits (2 sizes)
Storage	4°C, do not freeze
Capacity	Protein A/G: >7mg human IgG/mL resin Protein A/G UltraLink Resin: >20mg human IgG/mL resin Protein A/G Plus: >50mg human IgG/mL resin Protein A/G Plus on UltraLink Support: >28mg human IgG/mL resin Protein A/G Coated Plates: ~5pmol rabbit IgG/well

Ordering Information

Product #	Description	Pkg. Size
15138	Protein A/G, Clear 96-Well Plates	5 plates
20421	Protein A/G Agarose	3mL
20422	Protein A/G Agarose	15mL
89930	Pierce Chromatography Cartridges, Protein A/G	2 x 1mL
89931	Pierce Chromatography Cartridge, Protein A/G	1 x 5mL
89954	NAb Protein A/G Spin Columns	10 x 0.2mL
89958	NAb Protein A/G Spin Columns	5 x 1mL
89962	NAb Protein A/G Spin Column	1 x 5mL
89950	NAb Protein A/G Spin Purification Kit	Kit
89980	NAb Protein A/G Spin Purification Kit	Kit
53132	Protein A/G UltraLink Resin	2mL
53133	Protein A/G UltraLink Resin	10mL
21186	Pierce Recombinant Protein A/G	5mg
20423	Protein A/G Plus Agarose	2mL
53135	Protein A/G Plus on UltraLink Support	2mL

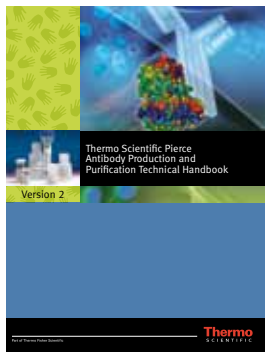
Protein L

Protein L Beads – Quick Reference	
Protein L	Recombinant protein expressed in *E. coli* (35.8kDa; four IgG-binding sites)
Specificity (Table 3)	Best for human or mouse monoclonal antibodies known to have appropriate kappa light chains; poor for general-purpose (polyclonal) IgG purification
Supports Offered	Crosslinked 6% beaded agarose
Package Formats	Resin slurries (2 sizes) Chromatography Cartridges (2 sizes) Packed Spin Columns (3 sizes) NAb IgG Purification Kits (2 sizes)
Storage	4°C, do not freeze
Capacity	Protein L: 5-10mg human IgG/mL resin Protein L Plus: >10-20mg human IgG/mL resin

Ordering Information

Product #	Description	Pkg. Size
20510	Protein L Agarose	2mL
20512	Protein L Agarose	10mL
89928	Pierce Chromatography Cartridges, Protein L	2 x 1mL
89929	Pierce Chromatography Cartridge, Protein L	1 x 5mL
89955	NAb Protein L Spin Columns	10 x 0.2mL
89959	NAb Protein L Spin Columns	5 x 1mL
89963	NAb Protein L Spin Column	1 x 5mL
89951	NAb Protein L Spin Purification Kit	Kit
89981	NAb Protein L Spin Purification Kit	Kit
21189	Pierce Recombinant Protein L	1mg
20520	Protein L Plus Agarose	2mL
15190	Protein L, Clear 96-Well Plates	5 plates

For complete product details, visit www.thermoscientific.com/pierce or request the Antibody Purification Handbook (1601974)



IgG Binding and Elution Buffers for Protein A, G, A/G and L

Binding and Elution Steps in Affinity Purification

Affinity purification procedures involving interaction of an antibody with its antigen generally use binding buffers at physiologic pH and ionic strength. However, many antibody purification methods do not use the antibody-antigen interaction; rather, they involve binding of antibodies by immobilized ligands that are not the antigen. In such cases, optimal binding conditions are determined by the unique properties of the antibody-ligand interaction, which may be different from physiologic pH and ionic strength.

Once the binding interaction occurs (i.e., the antibody is "captured" by the immobilized ligand), the support is washed with additional buffer to remove nonbound components of the sample. Finally, elution buffer is added to break the binding interaction and release the target molecule, which is then collected in its purified form. Elution buffer can dissociate binding partners by extremes of pH (low or high), high salt (ionic strength), the use of detergents or chaotropic agents that denature one or both of the molecules, removal of a binding factor, or competition with a counter ligand. In most cases, subsequent dialysis or desalting is required to exchange the purified protein from elution buffer into a more suitable buffer for storage or use.

Thermo Scientific Pierce IgG Binding and Elution Buffers have been optimized to provide the highest possible efficiency of IgG binding and elution using immobilized Protein A, Protein G and Protein A/G. Use of other buffer formulations may significantly alter not only the binding capacity but also the volumes of wash buffer required to ensure good purification.

Binding capacities with different Thermo Scientific Buffers expressed asmg of IgG bound per 2mL of resin.

	Immobilized Protein A		Immobilized Protein G		Immobilized Protein A/G	
Serum Sample	0.1 M Tris•HCl pH 8.0	Pierce Protein A Binding Buffer	0.1 M Tris•HCl pH 8.0	Pierce Protein G Binding Buffer	0.1 M Tris•HCl pH 8.0	Pierce Protein A Binding Buffer
Rabbit	17.81	33.19	21.51	27.75	13.89	19.61
Sheep	2.15	10.64	25.53	33.33	9.83	15.71
Bovine	6.16	22.76	31.72	48.10	15.13	22.06
Mouse	5.25	7.15	5.65	15.05	4.32	11.49
Rat	4.99	8.30	8.43	11.80	5.20	6.66
Horse	6.25	16.50	36.19	21.46	14.88	17.12
Dog	35.77	22.27	13.38	20.55	21.96	24.60
Chicken	0.91	1.21	1.63	7.27	1.21	4.10
Pig	29.61	24.83	21.25	27.51	19.24	29.48
Human	19.88	25.53	11.68	23.59	9.92	17.67

Ordering Information

Product #	Description	Highlights	Pkg. Size
54200	Protein A/G IgG Binding Buffer	• Ensures maximum recovery of IgG from immobilized Protein A/G	240mL
21001 21007	Protein A IgG Binding Buffer	• High-yield isolation of Mouse IgG_1 using Protein A columns • Premixed and easy to use	1L 3.75L
21019 21011	Protein G IgG Binding Buffer	• Ensures maximum recovery of IgG from immobilized Protein G	1L 3.75L
21004 21009	IgG Elution Buffer	• High-yield isolation of IgG from Immobilized Protein A and Protein G	1L 3.75L
21020 21012	Gentle Ag/Ab Binding Buffer pH 8.0	• Specially formulated and prefiltered • Eliminates use of harsh acidic elution conditions	1 L 3.75L
21030 21027 21013	Gentle Ag/Ab Elution Buffer pH 6.6	• Specially formulated for neutral elutions • Not compatible with phosphate buffers	100mL 500mL 3.75L
21016	IgM Binding Buffer	• Specially formulated for optimal binding of mouse IgM	800mL
21017	IgM Elution Buffer	• Specially formulated for optimal recovery of mouse IgM	500mL
21018	MBP Column Preparation Buffer	• Specially formulated for use with Immobilized MBP and IgM Purification Kit	50mL
21034	Mouse IgG_1 Mild Elution Buffer	• Separate IgG_1 from other IgG subclasses	500mL
21033	Mouse IgG_1 Mild Binding and Elution Buffer Kit Includes: Pierce Protein A IgG Binding Buffer Mouse IgG_1 Mild Elution Buffer Pierce IgG Elution Buffer	• Complete kit to allow mouse IgG_1 to be separated from other mouse IgG subclasses	Kit 1L 500mL 1L

Thermo Scientific Melon Gel Purification Products

Melon™ Gel Products provide an exciting new approach to purifying monoclonal and polyclonal antibodies from serum, tissue culture supernatant and ascites fluid. Melon Gel works with antibodies from a variety of species and subclasses, many of which do not purify efficiently with Protein A or Protein G. Because Melon Gel is not a bind-and-release support, it is extremely fast and gentle to your antibodies, resulting in antibody preparations of high purity and high activity!

How does it work?

Melon Gel contains a proprietary ligand that retains most protein found in serum, ascites and culture supernatants, while allowing IgG to pass through the support and be collected in the flow-through fraction. The resulting recovery and purity of the IgG isolated by this method rivals that obtained from the same samples using bind-and-release supports such as Protein A or Protein G.

Highlights:

- **Simple, one-step protocol** – no tedious binding, washing, and multiple elution steps
- **Rapid purification** – purifies antibodies from serum four to six times faster than Protein A or G methods
- **High recovery and purity** – antibodies from many species are recovered with greater than 90% yield and greater than 80% purity
- **Robust purification** – works with a wide range of antibodies including many that do not purify well on Protein A or Protein G
- **Gentle purification** – no harsh elution conditions means antibodies retain more activity
- **Reusable support** – Melon Gel Support can be used for multiple antibody purifications
- **Available in various formats** – spin columns, purification kits and chromatography cartridges for antibody purification from serum, ascites and culture supernatant

IgG purification performance of the Thermo Scientific Melon Gel System, Protein A and Protein G.

Source	Melon Gel	Protein A	Protein G
Human	H	H	H
Mouse	H	H	H
Rabbit	H	H	H
Rat	H	L	M
Goat	H	L	M
Cow	M	L	H
Sheep	M	L	H
Horse	H	L	H
Guinea Pig	H	H	L
Pig	H	H	L
Chicken	N	N	N
Hamster	H	M	M
Donkey	H	M	H

H = high recovery, M = medium recovery, L = low recover, N = no recovery

Ordering Information

Product #	Description	Pkg. Size
45206	Melon Gel IgG Spin Purification Kit	Kit
45212	Melon Gel IgG Spin Purification Kit	Kit
45214	Melon Gel Monoclonal IgG Purification Kit	Kit
45216	Saturated Ammonium Sulfate Solution	1L
45219	Ascites Conditioning Reagent	5mL
89932	Pierce Chromatography Cartridges, Melon Gel	2 x 1
89933	Pierce Chromatography Cartridges, Melon Gel	1 x 5
45208	Melon Gel Spin Kit Plate	2 plates
89972	Melon Gel Purification Buffer	1 pack
89973	Melon Gel Regenerant	1 pack

For more data using Melon Gel and for complete product information, please request the Antibody Purification Technical Handbook (1601974) or visit www.thermoscientific.com/pierce

Thiophilic Gel Antibody Purification

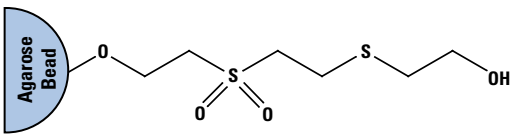
Thiophilic adsorption is a low-cost, efficient alternative to ammonium sulfate precipitation for immunoglobulin purification from crude samples. Ammonium sulfate precipitation must be followed by several additional steps to completely remove contaminants in crude samples. Thiophilic adsorption is a simple, rapid, one-step method for antibody purification from serum, ascites or tissue culture supernatant.

Thiophilic adsorption is a highly selective type of lyotropic salt-promoted protein:ligand interaction phenomenon that has been studied extensively by Porath and co-workers and other researchers.[1] This interaction is termed thiophilic because it distinguishes proteins that recognize a sulfone group in close proximity to a thioether. Thiophilic adsorption incorporates properties of both hydrophobic and hydrophilic adsorption. However, in contrast to strictly hydrophobic systems, thiophilic adsorption is not strongly promoted by high concentrations of sodium chloride. Instead, thiophilic adsorption is promoted by increased concentrations of water-interacting, non-chaotropic salts such as potassium and ammonium sulfate.

Thermo Scientific Pierce Thiophilic Adsorbent is 6% beaded agarose modified to contain simple sulfone/ thioether groups (see structure at right). Our Thiophilic Adsorbent has a high binding capacity (20mg of immunoglobulin permL of resin) and broad specificity toward immunoglobulins derived from various animal species. Notably, thiophilic adsorption is one of few methods available for purification of IgY from chicken (see also subsequent discussion of IgY purification). Among human serum proteins, immunoglobulins and α2-macroglobulins are preferentially bound by our Thiophilic Adsorbent.[2]

Purification using Pierce Thiophilic Adsorbent results in good protein recovery with excellent preservation of antibody activity. Sample preparation requires the addition of 0.5M potassium sulfate to the serum, ascites or culture fluid. Greater specificity for immunoglobulins is obtained if the sample is buffered at pH 8.0. The gentle elution conditions (e.g., 50mM sodium phosphate, pH 7-8) yield concentrated, essentially salt-free, highly purified immunoglobulins at near neutral pH.

After use, our Thiophilic Adsorbent can be regenerated by treatment with guanidine•HCl. Our data indicate that the Adsorbent column can be used at least 10 times without significant loss of binding capacity.



Structure of Thermo Scientific Pierce Thiophilic Adsorbent.

References
1. Porath, J., *et al.* (1985). *FEBS Lett.* **185,** 306-310.
2. Belew, M., *et al.* (1987). *J. Immunol. Method* **102,** 173-182.
3. Hutchens, T.W. and Porath, J. (1987). *Biochemistry* **26,** 7199-7204.
4. Lihme, A. and Heegaard, P.M.H. (1990). *Anal. Biochem.* **192,** 64-69.
5. Unpublished internal documents.

Thermo Scientific Pierce Thiophilic Adsorbent and Purification Kit

Economical purification of mouse antibodies from ascites fluid.

Highlights:

- Binds to Fab and F(ab´)2 fragments
- Binds to ScFv1
- High-capacity (20mg/mL), good protein recovery and retention of antibody function
- Broad specificity toward immunoglobulins derived from various animal species (see Table 4)
- Binds chicken IgY (also IgG)
- Simple, rapid, one-step purification for monoclonal antibodies from ascites; easy to scale up
- Used to enrich the immunoglobulin fraction from serum or tissue culture supernatant
- Efficient alternative to ammonium sulfate precipitation for enriching antibodies from crude samples
- Gentle elution conditions yield concentrated, salt-free immunoglobulin at near neutral pH
- High degree of purity

Table 4. Binding characteristics of Thermo Scientific Pierce Thiophilic Adsorbent.

Species	Total A_{280} Bound from 1mL Serum	% Purity by HPLC
Human	4.8	70
Mouse	8.6	63
Mouse IgG_1	11.6	92
Mouse IgG_{2a}	9.3	88
Mouse IgG_{2b}	9.8	97
Mouse IgG_3	10.7	94
Rat	13.0	79
Bovine	17.9	90
Calf	11.1	89
Chicken	5.2	76
Dog	12.2	91
Goat	17.3	92
Guinea Pig	11.1	71
Horse	13.0	93
Pig	21.1	90
Rabbit	6.7	84
Sheep	12.3	89

Reference
1. Schulze, R.A., *et al.* (1994). *Anal. Biochem.* **220,** 212-214.

Ordering Information

Product #	Description	Pkg. Size
20500	**Pierce Thiophilic Adsorbent**	10mL
44916	**Pierce Thiophilic Purification Kit**	Kit
	Includes: Thiophilic Adsorbent Columns	4 x 3mL
	Binding Buffer	1,000mL
	Elution Buffer	1,000mL
	Column Storage Buffer (2X)	100mL
	Guanidine•HCl Crystals	230g
	Column Extenders	

IgM Purification

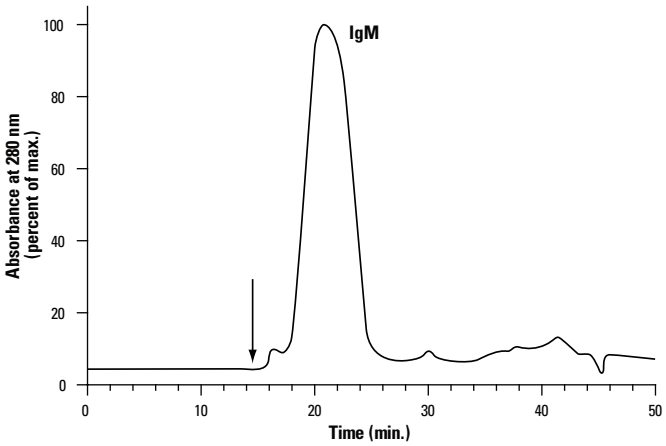
Immobilized Mannan Binding Protein

To develop an effective affinity matrix, our scientists examined C1q and another similarly structured protein, mannan binding protein (MBP). Serum MBP, like C1q, is capable of initiating carbohydrate-mediated complement activation. MBP is a mannose and *N*-acetylglucosamine-specific lectin found in mammalian sera, and it has considerable structural homology to C1q.[8] MBP subunits are identical, each with molecular mass of approximately 31kDa (C1q has six each of three different polypeptide subunits of molecular mass 24-28kDa). Studies in our labs show that MBP does not bind $F(ab')_2$ and Fab.

We have developed an easy-to-use Thermo Scientific Pierce Immobilized Mannan Binding Protein and Buffer System to purify IgM. It is most effective for purifying mouse IgM from ascites. Purified IgM can be obtained from a single pass over the affinity column. Human IgM will bind to the support, albeit with slightly lower capacity, and yield a product at least 88% pure as assessed by HPLC. The purification of IgM from other species and mouse serum has not yet been optimized.

Immobilized MBP and IgM Purification Kit

Easy IgM purification with guaranteed 88% pure mouse IgM!



Demonstration of the high purity of MBP-purified IgM from mouse ascites. The bound material from mouse ascites was eluted from the 5mL MBP column as described in the Standard Protocol. The highest 280nm absorbing fraction from the elution was chromatographed using the conditions described in the instructions.

Ordering Information

Product #	Description	Pkg. Size
22212	Immobilized Mannan Binding Protein Capacity: ~1mg IgM/mL of resin	10mL
44897	IgM Purification Kit	Kit
21016	IgM Binding Buffer	800mL
21017	IgM Elution Buffer	500mL
21018	MBP Column Preparation Buffer	50mL
53123	UltraLink Immobilized Mannan Binding Protein Capacity: >0.75mg IgM/mL of resin	5mL

IgA Purification

Human IgA Purification

Jacalin is an α-D-galactose binding lectin extracted from jackfruit seeds (*Artocarpus integrifolia*). The lectin is a glycoprotein of approximately 40kDa composed of four identical subunits. Jacalin immobilized on supports such as agarose has been useful for the purification of human serum or secretory IgA_1. IgA can be separated from human IgG and IgM in human serum or colostrum.[1] IgD is reported to bind to jacalin.[2] Immobilized jacalin is also useful for removing contaminating IgA from IgG samples.

Binding of IgA to immobilized jacalin occurs at physiologic pH and ionic strength, as in phosphate buffered saline (PBS). Elution of bound IgA occurs with competitor ligand (e.g., 0.1 M melibiose or 0.1 M α-D-galactose) in PBS. We offer immobilized jacalin on crosslinked 6% agarose.

References
1. Roque-Barreira, M.C. and Campos-Neto, A. (1985). *J. Immunol. Method* **134(30),** 1740-1743.
2. Aucouturier, P., *et al.* (1987). *Mol. Immunol.* **24(5),** 503-511.

Immobilized Jacalin

Ideal for human IgA purification.

Highlights:

- Ideal for preparing human IgA that is free of contaminating IgG
- Found to bind human IgA1, but not human IgA_2 – useful for separating the two subclasses

References
Kumar, G.S., *et al.* (1982). *J. Biosci.* **4,** 257-261.
Roque-Barreira, M.C. and Campos-Neto, A. (1985). *J. Immunol.* **134,** 1740-1743.
Mestecky, J., *et al.* (1971). *J. Immunol.* **107,** 605-607.
Van Kamp, G.J. (1979). *J. Immunol. Method* **27,** 301-305.
Kondoh, H., *et al.* (1986). *J. Immunol. Method* **88,** 171-173.

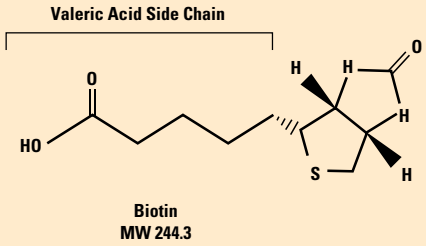
Ordering Information

Product #	Description	Pkg. Size
20395	Immobilized Jacalin Capacity: 1-3mg human IgA/mL of resin Support: Crosslinked 6% beaded agarose Loading: 4.5mg of jacalin/mL of resin	5mL

Avidin:Biotin Binding

Biotin

Biotin, also known as vitamin H, is a small molecule (MW 244.3) that is present in tiny amounts in all living cells. The valeric acid side chain of the biotin molecule can be derivatized to incorporate various reactive groups that are used to attach biotin to other molecules. Once biotin is attached to a molecule, the molecule can be affinity-purified using an immobilized version of any biotin-binding protein. Alternatively, a biotinylated molecule can be immobilized through interaction with a biotin-binding protein, then used to affinity-purify other molecules that specifically interact with it. We offer biotin-labeled antibodies and a number of other biotinylated molecules, as well as a broad selection of biotinylation reagents to label any protein.



Biotin
MW 244.3

Biotin-Binding Proteins

Avidin – The extraordinary affinity of avidin for biotin allows biotin-containing molecules in a complex mixture to be discretely bound with avidin. Avidin is a glycoprotein found in the egg white and tissues of birds, reptiles and amphibians. It contains four identical subunits having a combined mass of 67,000–68,000 daltons. Each subunit consists of 128 amino acids and binds one molecule of biotin. The extent of glycosylation on avidin is high; carbohydrate accounts for about 10% of the total mass of the tetramer. Avidin has a basic isoelectric point (pI = 10–10.5) and is stable over a wide range of pH and temperature. Extensive chemical modification has little effect on the activity of avidin, making it especially useful for protein purification. However, because of its carbohydrate content and basic pI, avidin has relatively high nonspecific binding properties.

Streptavidin – Another biotin-binding protein is streptavidin, which is isolated from *Streptomyces avidinii* and has a mass of 75,000 daltons. In contrast to avidin, streptavidin has no carbohydrate and has a mildly acidic pI (5.5). Thermo Scientific Pierce Streptavidin is a recombinant form having a mass of 53,000 daltons and a near-neutral pI. Streptavidin is much less soluble in water than avidin. There are considerable differences in the composition of avidin and streptavidin, but they are remarkably similar in other respects. Streptavidin is also a tetrameric protein, with each subunit binding one molecule of biotin with affinity similar to that of avidin. Guanidinium chloride will dissociate avidin and streptavidin into subunits, but streptavidin is more resistant to dissociation. Streptavidin contains an RYD sequence similar to the RGD sequence that binds cell surface receptors. The RYD sequence can cause background in some applications.

NeutrAvidin Protein – We also offer a deglycosylated version of avidin, known as NeutrAvidin Protein, with a mass of approximately 60,000 daltons. As a result of carbohydrate removal, lectin binding is reduced to undetectable levels, yet biotin-binding affinity is retained because the carbohydrate is not necessary for this activity. NeutrAvidin Protein offers the advantages of a near-neutral pI (6.3) to minimize nonspecific adsorption, along with lysine residues that remain available for derivatization or conjugation. NeutrAvidin Protein yields the lowest nonspecific binding among the known biotin-binding proteins due to its near-neutral pI and lack of both carbohydrate and RYD sequence.

A Comparison of Biotin-Binding Proteins

The strong association between avidin and biotin can be used in the field of affinity separations. By attaching avidin to a solid support, a biotinylated product can be anchored to the same solid support. The attachment is stable over a wide range of pH, salt concentrations and temperatures. To dissociate biotin from avidin, 8M guanidine•HCl, pH 1.5 or boiling in SDS-PAGE sample buffer must be used.

	Avidin	Streptavidin	Thermo Scientific NeutrAvidin Protein
Molecular Weight	67K	53K	60K
Biotin-binding Sites	4	4	4
Isoelectric Point (pI)	10	6.8–7.5	6.3
Specificity	Low	High	Highest
Affinity for Biotin (K_d)	10^{-15}M	10^{-15}M	10^{-15}M
Nonspecific Binding	High	Low	Lowest

Immobilized Avidin Products

Strong biotin interaction creates a nearly irreversible bond.

Immobilized avidin can be used in a variety of applications for the affinity purification of biotinylated macromolecules. In one variation, an antibody that has an affinity for a particular antigen is labeled with biotin. Cells containing the antigen are lysed, then incubated with the biotinylated antibody to form a typical antigen/antibody complex. To isolate the antigen, the crude mixture is passed through an immobilized avidin or streptavidin column, which will bind the complex. After appropriate washes, the antigen can be eluted from the column with a low pH elution buffer. The biotinylated antibody is retained by the column.

Applications:

- Binding biotinylated anti-transferrin for purifying transferrin from serum[1]
- Binding biotinylated peptides and elution with an SDS/urea solution[2]
- Hybridization of biotinylated RNA to its complementary DNA and binding to immobilized avidin, with subsequent elution of the single-stranded DNA[3]
- Purification of double-stranded DNA[4]

References

1. Wilchek, M. and Bayer, E.A. (1989). *Protein Recognition of Immobilized Ligands. Hutchins*, T.W., ed. Alan R. Liss, Inc., pp. 83–90.
2. Swack, J.A., *et al.* (1978). *Anal. Biochem.* **87,** 114–126.
3. Manning, J., *et al.* (1977). *Biochemistry* **16,** 1364–1370.
4. Pellegrini, M., *et al.* (1977). *Nucleic Acids Res.* **4,** 2961–2973.

Ordering Information

Product #	Description	Pkg. Size
20219	**Avidin Agarose Resin** Support: Crosslinked 6% beaded agarose Capacity: ≥20µg biotin/mL resin	5mL
20225	**Avidin Agarose Resin** Support and Capacity: Same as above	5 x 5mL
20362	**Avidin Agarose Columns** Support and Capacity: Same as above	5 x 1mL

Immobilized Streptavidin Products

Same high biotin-binding affinity as avidin with low nonspecific binding.

Applications:

- Purification of membrane antigens in conjunction with biotinylated monoclonal antibodies[1,2]
- Cell-surface labeling with biotinylation reagents, followed by precipitation with immobilized streptavidin[3]
- Purification of cell-surface glycoproteins using biotinylated Concanavalin A[4]
- Recovery of single-stranded DNA for dideoxy sequencing[5]

References

1. Gretch, D.R., *et al.* (1987). *Anal. Biochem.* **163,** 270–277.
2. Updyke, T.V. and Nicolson, G.L. (1984). *J. Immunol. Method* **73,** 83–95.
3. Lisanti, M.P., *et al.* (1989). *J. Cell Biol.* **109,** 2117–2127.
4. Buckie, J.W. and Cook, G.M. (1986). *Anal. Biochem.* **156(2),** 463–472.
5. Baqui, M., *et al.* (2003). *J. Biol. Chem.* **278,** 1206–1211.

Ordering Information

Product #	Description	Pkg. Size
20347	**Streptavidin Agarose Resin** Support: Crosslinked 6% beaded agarose Capacity: 1–3mg biotinylated BSA/mL resin 15–28µg biotin/mL resin	2mL
20349	**Streptavidin Agarose Resin** Support and Capacity: Same as above	5mL
20353	**Streptavidin Agarose Resin** Support and Capacity: Same as above	10mL
20351	**Streptavidin Agarose Columns** Support and Capacity: Same as above	5 x 1mL
53113	**Streptavidin UltraLink Resin** Support: UltraLink Biosupport Capacity: ≥2mg biotinylated BSA/mL resin ≥24µg biotin/mL resin	2mL
53114	**Streptavidin UltraLink Resin** Support and Capacity: Same as above	5mL
53116	**Streptavidin Plus UltraLink Resin** Support: UltraLink Biosupport Capacity: ≥4mg biotinylated BSA/mL resin ≥48µg biotin/mL resin	2mL
53117	**Streptavidin Plus UltraLink Resin** Support and Capacity: Same as above	5mL
20357	**High Capacity Streptavidin Agarose Resin** Support: Crosslinked 6% beaded agarose Capacity: >10mg biotinylated BSA/mL of resin	2mL
20359	**High Capacity Streptavidin Agarose Resin** Support and Capacity: Same as above	5mL
20361	**High Capacity Streptavidin Agarose Resin** Support and Capacity: Same as above	10mL
21344	**MagnaBind Streptavidin Beads** Support: 1–4µm, iron oxide particles Capacity: 2µg biotin/mL beads	5mL
88816	**Pierce Streptavidin Magnetic Beads**	1mL
88817	**Pierce Streptavidin Magnetic Beads**	5mL

Thermo Scientific Products for Avidin:Biotin Binding

Immobilized NeutrAvidin Products

Less nonspecific binding produces cleaner results and better yields.

When nonspecific binding is a problem in your application, Thermo Scientific Immobilized NeutrAvidin Products are superior alternatives to avidin or streptavidin. NeutrAvidin Biotin-Binding Protein is a modified avidin derivative that combines several key features to provide biotin-binding with exceptionally low nonspecific binding properties.

Highlights:

- Carbohydrate-free – just like streptavidin, NeutrAvidin Biotin-Binding Protein has no carbohydrate, eliminating nonspecific binding problems due to sugars
- No interaction with cell surface molecules – absence of the Arg-Tyr-Asp sequence (present in streptavidin), which mimics the universal cell surface recognition sequence present in a variety of molecules, eliminates cross-reactivity of cell surface molecules
- Neutral pI – with a pI of 6.3, NeutrAvidin Protein has a pI that is closer to neutrality than avidin or streptavidin, eliminating electrostatic interaction that contributes to nonspecific binding

Applications:

- Immunoprecipitation
- Purifying proteins that bind to biotinylated ligands
- Capturing biotinylated cell-surface proteins[1-3]
- Purifying biotinylated peptides[4]

Ordering Information

Product #	Description	Pkg. Size
29200	**NeutrAvidin Agarose Resin** Support: Crosslinked 6% beaded agarose Capacity: >20µg or 80nmol biotin/mL resin (approx. 1–2mg biotinylated BSA/mL resin)	5mL
29201	**NeutrAvidin Agarose Resin** Support and Capacity: Same as above	10mL
53150	**NeutrAvidin UltraLink Resin** Support: UltraLink Biosupport Capacity: 12–20µg biotin/mL gel	5mL
53151	**NeutrAvidin Plus UltraLink Resin** Support: UltraLink Biosupport Capacity: ≥30µg biotin/mL gel	5mL
29202	**High Capacity NeutrAvidin Agarose Resin** Support: Crosslinked 6% beaded agarose Capacity: >75µg biotin/mL resin >8mg biotinylated BSA/mL resin	5mL
29204	**High Capacity NeutrAvidin Agarose Resin** Support and Capacity: Same as above	10mL

Immobilized Monomeric Avidin and Kit

Ideal affinity support for gentle, reversible binding of biotinylated proteins.

To break the avidin-biotin interaction, 8M guanidine•HCl at pH 1.5 or boiling in SDS-PAGE sample buffer is required. These elution methods may result in denaturation of the biotinylated protein and cause irreversible damage to the support. In addition, avidin or streptavidin will be irreversibly denatured and lose the ability to bind subsequent biotinylated samples.

When avidin is coupled to a solid support as the subunit monomer, the specificity for biotin is retained, but the affinity for biotin binding substantially decreases (K_a~10^8 M^{-1}). The Monomeric Avidin Agarose Resin and Kit can be used to bind biotinylated molecules, and the bound material can be competitively eluted using 2mm biotin in phosphate-buffered saline (PBS). This technique provides the gentlest elution conditions without contamination of the avidin subunits or substantial loss of column-binding capacity.

Highlights:

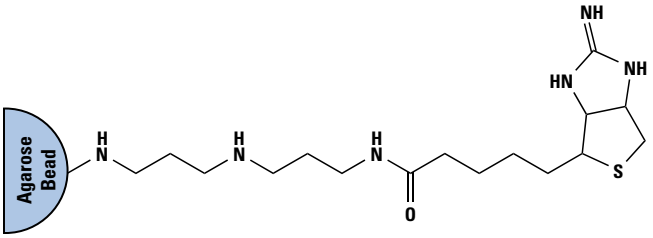
- Purifies biotinylated products under mild elution conditions
- Can be regenerated and reused at least 10 times
- Exhibits little nonspecific binding (3% or less)

Ordering Information

Product #	Description	Pkg. Size
20228	**Monomeric Avidin Agarose Resin** Support: Crosslinked 4% beaded agarose Capacity: ≥1.2mg biotinylated BSA/mL resin	5mL
20267	**Monomeric Avidin Agarose Resin** Support and Capacity: Same as above	10mL
20227	**Monomeric Avidin Agarose Kit** Support and Capacity: Same as above Includes: 1 x 2mL Column, Binding and Elution buffers	Kit
53146	**Immobilized Monomeric Avidin UltraLink Resin** Support: UltraLink Biosupport Capacity: ≥1.2mg biotinylated BSA/mL resin	5mL
29129	**Biotin**	1g

Immobilized Iminobiotin and Biotin

Iminobiotin offers mild dissociation conditions at pH 4.



Immobilized Iminobiotin

Iminobiotin is the guanido analog of biotin. The dissociation constant of the avidin-iminobiotin complex is pH-dependent. At pH 9.5-11.0, the avidin-iminobiotin complex will bind tightly. At pH 4, the avidin-iminobiotin complex will dissociate. Because denaturing agents such as 8 M guanidine•HCl or 4 M urea are not used in the purification, an avidin conjugate has a better chance of maintaining its activity during purification.

Use immobilized D-Biotin as an "irreversible linkage" to bind streptavidin conjugates. The biotin-streptavidin interaction can withstand extremes in pH, salt and detergents.

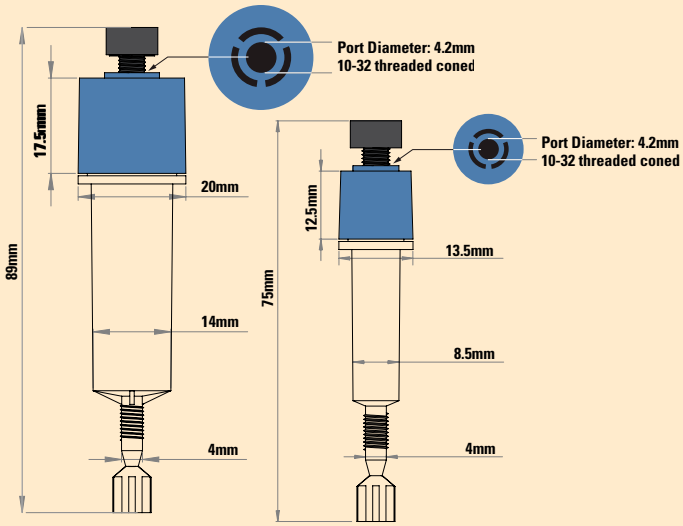
Ordering Information

Product #	Description	Pkg. Size
20221	**Iminobiotin Agarose Resin** Support: Crosslinked 6% beaded agarose Spacer: Diaminodipropylamine Capacity: ≥1mg of avidin/mL resin	5mL
20218	**Biotin Agarose Resin** Support: Pierce CDI Support Spacer: Diaminodipropylamine Capacity: ≥2mg of avidin/mL resin	5mL

FPLC Cartridge Overview

Thermo Scientific Pierce Chromatography Cartridges are convenient, reliable, ready-to-use, pre-packed 1mL and 5mL columns of sample-prep and affinity purification resins for manual or automated liquid chromatography (LC).

The cartridge fittings are compatible with the popular automated liquid-chromatography systems or for manual syringe processing. The cartridges attach directly to ÄKTA or FPLC Systems without additional connectors. Cartridges can be used individually or connected in a series to obtain even higher column capacity. Each product supplied in the Pierce Chromatography Cartridge format accessory pack that readily adapts cartridges for use using Luer-Lok Syringe Fittings or tubing. The cartridges provide fast, easy and reproducible chromatographic separations and can be regenerated for multiple uses.



Thermo Scientific FPLC Cartridges shematic.

Chromatography Cartridge Highlights

- **Two sizes** – 1mL and 5mL, convenient for typical research scales
- **Compatible** – fittings allow connection with popular LC systems or a standard syringe
- **Versatile** – use singly or connected in series to service different capacity requirements
- **Validated** – available cartridges have been tested to ensure performance in the format
- **Reusable** – accessory pack includes caps for convenient storage between uses
- **Economical** – comparable performance at lower cost than other commercially available cartridges

Thermo Scientific Pierce Chromatography Cartridge properties.

	1mL Cartridge	5mL Cartridge
Dimensions	0.7 x 2.7cm	1.3 x 3.8cm
Recommended Flow Rate	1-4mL/min	1-7mL/min
Maximum Pressure	0.3 mPa (43 psi or 3 bar)	0.3 mPa (43 psi or 3 bar)
Cartridge Material	polypropylene	polypropylene
Frit Material	polyethylene	polyethylene

Applications for Pierce Chromatography Cartridges:

- His-tagged protein purification
- GST-tagged protein purification
- Antibody purification
- Biotin binding
- Phosphoprotein enrichment
- Protein desalting

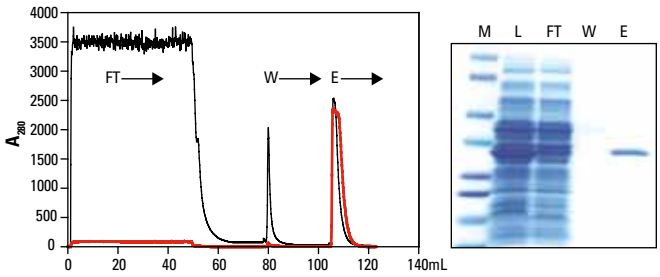
His-tagged Protein FPLC Purification

Thermo Scientific HisPur Nickel-NTA Cartridges

Delivers the highest yield of His-tagged protein with a re-useable resin.

Highlights:

- Bind up to 60mg of 6xHis-tagged protein per milliliter of resin
- Purify proteins using native or denaturing conditions
- Use with Pierce Cell Lysis Reagents and a variety of buffer additives
- Reuse cartridges several times



Purification of 6xHis-GFP from *E. coli* lysate using a Thermo Scientific HisPur Nickel-NTA Cartridge. Bacterial lysate (130mg total protein) containing over-expressed 6xHis-GFP (green fluorescent protein) was diluted 1:1 with equilibration buffer and applied to a HisPur Ni-NTA Chromatography Cartridge at a flow rate of 1mL/min. The cartridge was washed with PBS, 68mM imidazole until the baseline absorbance was reached. The 6xHis-GFP was eluted with PBS, 300mM imidazole. **Left panel:** 6xHis-GFP elution was monitored at 280nm (black line) and 485nm (red line; GFP-specific). **Right panel:** Selected fractions were analyzed by SDS-PAGE. Gel lanes were normalized to equivalent volume. **M =** MW marker, **L =** lysate load, **FT =** flow-through and **E =** elution.

See page 14 for more information and other packaging formats of HisPur Ni-NTA Resin.

Ordering Information

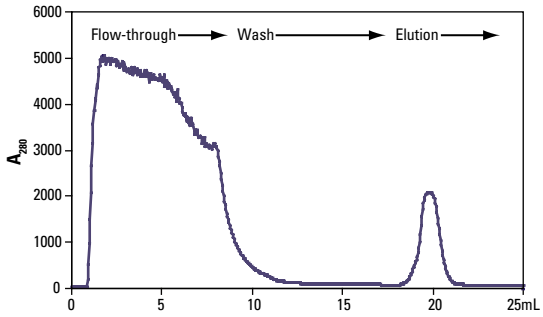
Product #	Description	Pkg. Size
90098	**HisPur Ni-NTA Chromatography Cartridges, 1mL** Formulation: 1mL resin cartridges with LC and Luer-Lok fittings. *Sufficient for: Binding up to 60mg of His-tagged protein per cartridge.*	5 cartridges
90099	**HisPur Ni-NTA Chromatography Cartridges, 5mL** Formulation: 5mL resin cartridges with LC and Luer-Lok fittings. *Sufficient for: Binding up to 300mg of His-tagged protein per cartridge.*	2 cartridges

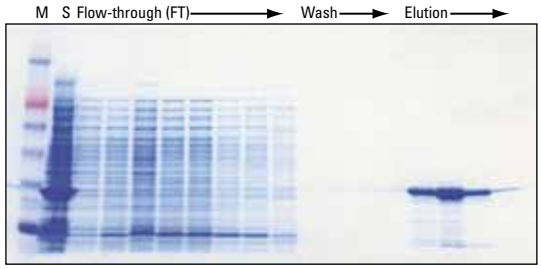
Thermo Scientific HisPur Cobalt Cartridges

Highly selective binding for the highest purity His-tagged protein purification.

Highlights:

- Obtain more than 10mg of pure His-tagged protein per milliliter of resin without optimizing imidazole washing conditions
- Cobalt-chelate coordination core binds fewer host protein contaminants, resulting in lower background than nickel resins
- No metal contamination in eluted histidine-tagged protein sample
- Purify proteins under native or denaturing conditions; compatible with Pierce Cell Lysis Reagents and a variety of buffer additives
- Reuse cartridges several times





Purification of 6xHis-GFP from *E. coli* lysate using a Thermo Scientific HisPur Cobalt Cartridge. His-tagged green fluorescent protein (GFP) was extracted from *E. coli* using Thermo Scientific B-PER Bacterial Protein Extraction Reagent in Phosphate Buffer (Product # 78266) containing Thermo Scientific Halt Protease Inhibitor Cocktail, EDTA-Free (Product # 78415). The lysate was diluted 1:1 with equilibration/wash buffer (50mM sodium phosphate, 300mM sodium chloride, 10mM imidazole, pH 7.4) and applied to a HisPur Cobalt Chromatography Cartridge at a flow rate of 0.3mL/min. The cartridge was washed with equilibration/wash buffer until the baseline absorbance at 280nm was reached. His-tagged GFP was eluted (50mM sodium phosphate, 300mM sodium chloride, 150mM imidazole; pH 7.4) and selected fractions were analyzed by SDS-PAGE and Thermo Scientific GelCode Blue Stain Reagent (Product # 24592). **M =** MW Marker; S = non-fractionated lysate; **FT =** flow-through.

See page 16 for more information and other packaging formats of HisPur Ni-Cobalt Resin.

Ordering Information

Product #	Description	Pkg. Size
90093	**HisPur Cobalt Chromatography Cartridges, 1mL** Formulation: 1mL resin cartridges with LC and Luer-Lok fittings. *Sufficient for: Binding > 10mg of His-tagged protein per cartridge.*	5 cartridges
90094	**HisPur Cobalt Chromatography Cartridges, 5mL** Formulation: 5mL resin cartridges with LC and Luer-Lok fittings. *Sufficient for: Binding > 50mg of His-tagged protein per cartridge.*	2 cartridges

GST-Tagged Protein FPLC Purification

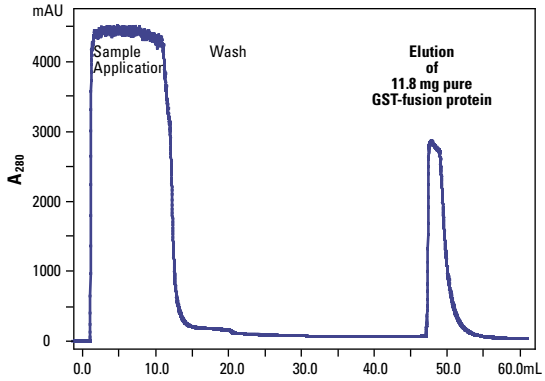
Thermo Scientific Pierce Glutathione Agarose Cartridges

Cost-effective and high-performance GSH resins for purification of recombinant GST fusion proteins.

Highlights:

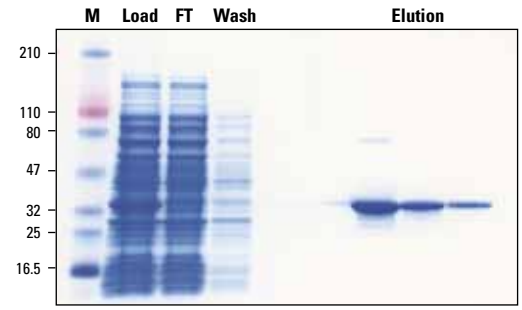
- Binds at least 25mg of recombinant GST protein per milliliter of resin
- Consistently purifies at least 10mg of GST-tagged protein per milliliter of resin with greater than 90% purity
- Economically priced and can be reused at several times without reduction in binding capacity and purification performance
- Works well to purify GST-fusion proteins from bacterial lysates or use with pre-purified GST-tagged proteins to pull down protein interactions
- Validated and effective for use with Pierce Cell Lysis Reagents to extract and purify from bacterial or mammalian cell cultures

See page 18 for more information and other packaging formats of Pierce Immobilized Glutathione Agarose Resin.



Purification of Pak 1-GST fusion protein on Thermo Scientific Pierce Glutathione Chromatography Cartridge 1mL.

Sample: 50mg (10mL) clarified *E. coli* lysate containing expressed GST-Pak 1, M 34,000
Binding Buffer: 5mM Tris, 150mM sodium chloride, pH 8.0
Elution Buffer: 50mM Tris, 150mM sodium chloride, 10mM Glutathione, pH 8.0
Flow Rate: Load at 0.5mL/min, Wash, Elute 1mL/min
Instrument: AKTApurifier



Purification of Pak 1-GST fusion protein using the Pierce GST Spin Purification Kit (Product # 16106). Pak 1-GST lysate (2.4mg total protein) was applied in Glutathione Binding Buffer to a 0.2mL Pierce Glutathione Spin Column and eluted with 10mM Glutathione Elution Buffer, pH 8.0. Fractions were resolved by SDS-PAGE using a 4-20% Tris-Glycine gel. Gel was stained with GelCode Blue Stain Reagent (Product #24590). Pierce 3-Color Protein Molecular Weight Marker Mix (Product # 26691) was used.

Ordering Information

Product #	Description	Pkg. Size
16109	**Pierce Glutathione Chromatography Cartridges, 1mL** Formulation: 1mL resin cartridges with LC and Luer-Lok fittings. *Sufficient for: Binding approx. 10mg of GST-tagged protein per cartridge.*	5 cartridges
16110	**Pierce Glutathione Chromatography Cartridges, 5mL** Formulation: 5mL resin cartridges with LC and Luer-Lok fittings. *Sufficient for: Binding approx. 50mg of GST-tagged protein per cartridge.*	2 cartridges

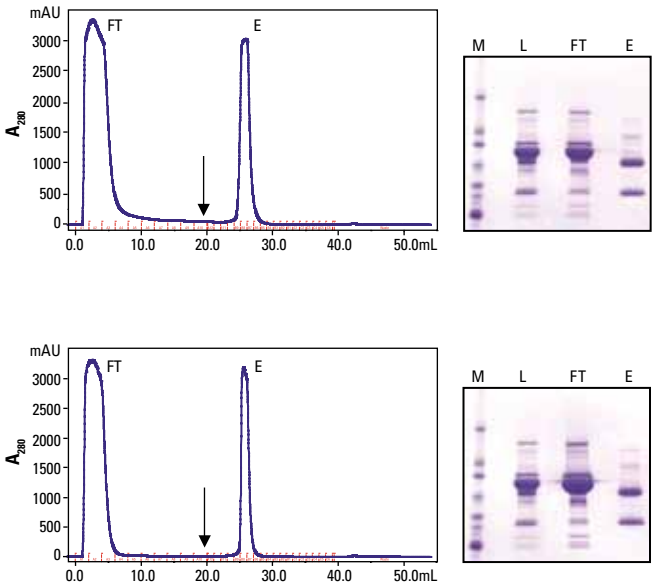
Antibody FPLC Purification

Thermo Scientific Pierce Protein A Agarose Cartridges

Ideal for purification of IgG from serum and other fluids.

Highlights:

- Binds to a wide range of antibodies – especially good for purification of rabbit IgG
- Less expensive than Protein G agarose



Comparable antibody yield and purity achieved with Thermo Scientific Pierce Protein A Chromatography Cartridge. Normal human serum (60mg) was applied in PBS to a 1mL Thermo Scientific Pierce Protein A Cartridge (top) and a HiTrap® Column (bottom) and eluted with 0.1M glycine, pH 2.8, using a flow rate of 1mL/minute. The arrow denotes the start of the low-pH elution. The yield of human IgG was 6.85mg and 6.88mg, respectively. Fractions were separated by SDS-PAGE and the gels were stained with Thermo Scientific Imperial Protein Stain (Product # 24615). **M =** MW marker, **L =** sample load, **FT =** flow-through and **E =** elution.

See page 60 for more information and other packaging formats of Pierce Protein A Agarose Resin.

Ordering Information

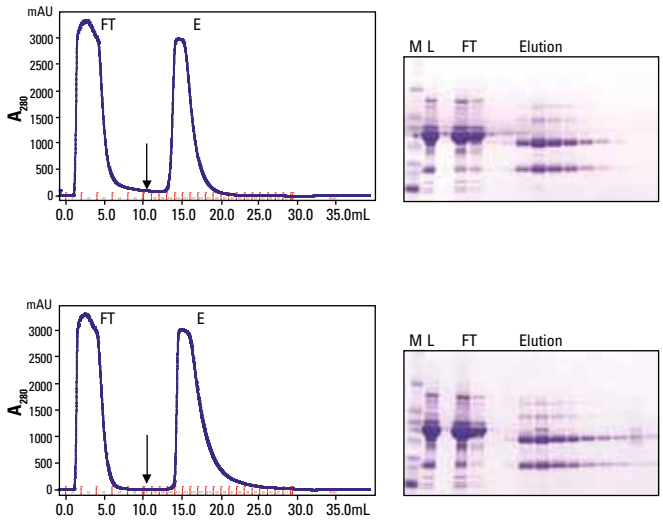
Product #	Description	Pkg. Size
89924	**Pierce Protein A Chromatography Cartridges, 1mL** Formulation: 1mL resin cartridges with LC and Luer-Lok fittings. *Sufficient for: Binding 35mg human IgG per cartridge.*	2 cartridges
89925	**Pierce Protein A Chromatography Cartridges, 5mL** Formulation: 5mL resin cartridge with LC and Luer-Lok fittings. *Sufficient for: Binding 175mg human IgG per cartridge.*	1 cartridge

Thermo Scientific Pierce Protein G Agarose Cartridges

Especially suited for purification of monoclonal antibodies from mouse and the broadest spectrum of species and IgG subclasses from human, goat and sheep samples.

Highlights:

- Albumin and cell surface binding site have been removed from Protein G to provide higher antibody purity
- Binds to a wider range of antibodies than Protein A beads



Comparable antibody yield and purity acheived with Thermo Scientific Pierce Protein G Chromatography Cartridge. Normal human serum (60mg) was applied in PBS to a 1mL Thermo Scientific Pierce Protein G Cartridge (top) and a 1mL HiTrap Column (bottom) and eluted with 0.1M glycine, pH 2.8, using a flow rate of 1mL/minute. The arrow denotes the start of the low pH elution. Fractions were separated by SDS-PAGE and the gels were stained with Imperial® Protein Stain (Product # 24615). **M =** MW marker, **L =** sample load, **FT =** flow-through and Elution = elution fractions.

See page 60 for more information and other packaging formats of Pierce Protein G Agarose Resin

Ordering Information

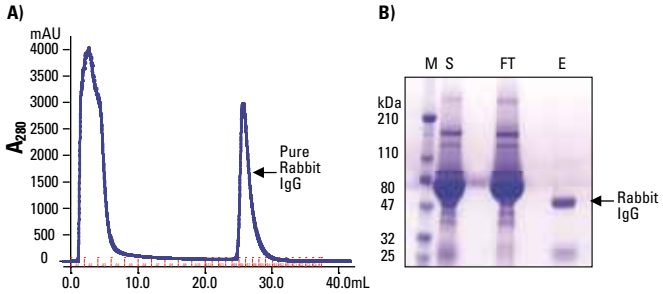
Product #	Description	Pkg. Size
89926	**Pierce Protein G Chromatography Cartridges, 1mL** Formulation: 1mL resin cartridges with LC and Luer-Lok Fittings. *Sufficient for: Binding 11 to 15mg human IgG per cartridge.*	2 cartridges
89927	**Pierce Protein G Chromatography Cartridges, 5mL** Formulation: 5mL resin cartridge with LC and Luer-Lok Fittings. *Sufficient for: Binding 55 to 75mg human IgG per cartridge.*	1 cartridge

Thermo Scientific Pierce Protein A/G Agarose Cartridges

Genetically-engineered protein that combines the IgG binding domains of both Protein A and Protein G.

Highlights:

- Single support provides all benefits of Protein A and Protein G
- Binds a wider range of antibodies than Protein A and Protein G beads



Thermo Scientific Pierce Protein A/G Chromatography Cartridges are effective for affinity purification of immunoglobins from serum. A) 2mL of rabbit serum was applied to the cartridge and the resulting chromatogram recorded. **B)** Fractions were analyzed by SDS-PAGE on a 4-20% Tris Glycine gel stained with Imperial Protein Stain (Product # 24615). M= Marker proteins, S= Sample applied, FT= Flow-through during sample load and wash, E= Eluted rabbit IgG. The arrow indicates the location of the isolated IgG.

See page 61 for more information and other packaging formats of Pierce Protein A/G Agarose Resin.

Ordering Information

Product #	Description	Pkg. Size
89930	**Pierce Protein A/G Chromatography Cartridges, 1mL** Formulation: 1mL resin cartridges with LC and Luer-Lok Fittings. *Sufficient for: Binding 7mg human IgG per cartridge.*	2 cartridges
89931	**Pierce Protein A/G Chromatography Cartridges, 5mL** Formulation: 5mL resin cartridge with LC and Luer-Lok Fittings. *Sufficient for: Binding 35mg human IgG per cartridge.*	1 cartridge

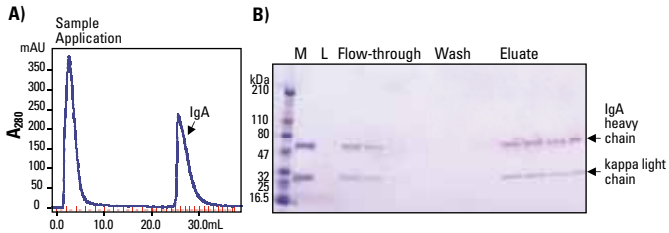
Thermo Scientific Pierce Protein L Agarose Cartridges

Great for purifying ScFv or Fab fragments and monoclonal antibodies containing kappa light chains.

Highlights:

- Binds kappa light chains from a wide range of species without interfering with antigen-binding sites†
- Binds to all classes of Ig (e.g., IgG, IgM, IgA, IgE and IgD)†
- Binds single-chain variable fragments (ScFv)†
- Does not bind bovine, goat or sheep Igs
- Does not bind to bovine antibodies, making it ideal for purification of mouse IgG† from cell culture supplemented with bovine serum

†**Note:** *Lambda light chains and some kappa light chains will not bind. Binding will only occur if the appropriate kappa light chains are present.*



Thermo Scientific Pierce Protein L Chromatography Cartridges isolate and purify immunoglobulin classes IgG, IgM, IgA, IgE and IgD via their kappa light chains. A) Human IgA Serum (2mg) was applied in 100mM sodium phosphate, 150mM sodium chloride, pH 7.2 to a 1mL Pierce Protein L Chromatography Cartridge and purified at a flow rate of 1mL/minute. Target was eluted in 0.1M glycine, pH 2.8. **B)** Fractions were analyzed by SDS-PAGE on a 4-20% Tris-Glycine gel stained with GelCode Blue Stain Reagent (Product # 24590).

See page 61 for more information and other packaging formats of Pierce Protein L Agarose Resin.

Ordering Information

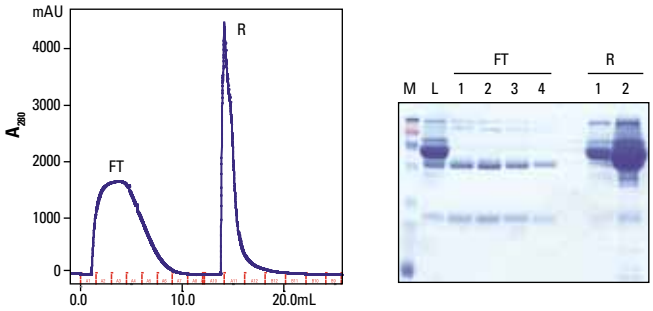
Product #	Description	Pkg. Size
89928	**Pierce Protein L Chromatography Cartridges, 1mL** Formulation: 1mL resin cartridges with LC and Luer-Lok fittings. *Sufficient for: Binding 5 to 10mg human IgG per cartridge.*	2 cartridges
89929	**Pierce Protein L Chromatography Cartridges, 5mL** Formulation: 5mL resin cartridge with LC and Luer-Lok Fittings. *Sufficient for: Binding 25 to 50mg human IgG per cartridge.*	1 cartridge

Thermo Scientific Melon Gel IgG Purification Resins

Easily purify IgG from serum in 15 minutes.

Highlights:

- No tedious binding, washing and multiple elution steps
- Purifies antibodies from serum four to six times faster than Protein A or G methods
- Recover antibodies from many species with >80% purity
- No harsh elution conditions means antibodies retain more activity
- Reusable support



Effective reverse-affinity purfication of antibody with Thermo Scientific Melon Gel Cartridges. Normal human serum (1mL) was applied to a 1mL Melon Gel Cartridge at a flow rate of 1mL/minute. Protein contaminants bind to the resin while the antibodies flow through (FT). Volume of recovered antibody = 4 to 5mL. Regeneration (R) solution strips the bound protein contaminants so that the cartridge can be reused multiple times. The purity of the isolated antibody was evaluated by SDS-PAGE (right panel) and stained with Thermo Scientific GelCode Blue Stain Reagent (Product # 24590). **M =** MW Marker, **L =** sample loaded, **FT =** flow-through and **R =** regeneration fractions.

See page 63 for more information and other packaging formats of Melon Gel Resin.

Ordering Information

Product #	Description	Pkg. Size
89932	**Melon Gel Chromatography Cartridges, 1mL** Formulation: 1mL resin cartridges with LC and Luer-Lok Fittings. *Sufficient for: IgG purification from 1 to 2mL serum per cartridge.*	2 cartridges
89933	**Melon Gel Chromatography Cartridges, 5mL** Formulation: 5mL resin cartridge with LC and Luer-Lok Fittings. *Sufficient for: IgG purification from 5 to 10mL serum per cartridge.*	1 cartridge

Phosphoprotein FPLC Purification

Thermo Scientific Pierce Phosphoprotein Enrichment Kit

Purify and isolate phosphoproteins for analysis by Western blotting or mass spectrometry.

Highlights:

- Low nonspecific protein contamination from complex biological samples, such as cell culture lysate and mouse tissue extract
- Easy spin format enables enrichment of phosphorylated proteins in less than 2 hours
- Achieves higher yields than other commercially available kits

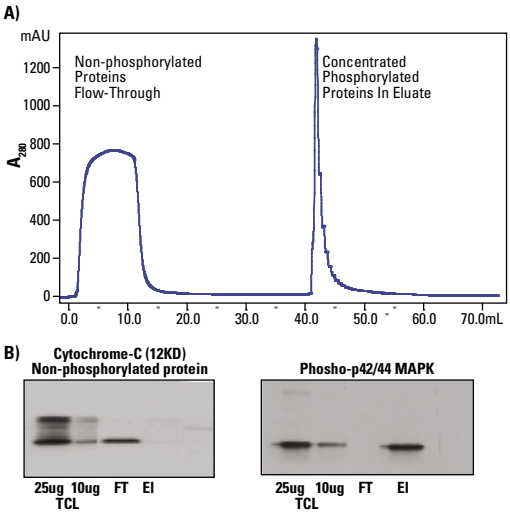


Figure A. Enrichment of phosphorylated proteins from K562 cell lysate, 4mg (10mL), processed using a Thermo Scientific Pierce Phosphoprotein Enrichment Chromatography Cartridge, 1mL. Binding/Wash Buffer: 50mM MES 2-(N-morpholino) ethanesulfonic acid, monohydrate), 250mM sodium chloride, 25mM adipic acid, 0.25% CHAPS; pH 5.0; Elution Buffer: 100mM sodium phosphate, 500mM sodium chloride, 0.25% CHAPS; pH 7.5; Flow rate during sample application 0.3mL/min; 1mL/min during wash and elution.
Figure B. Thermo Scientific Pierce Phosphoprotein Enrichment Chromatography Cartridge, 1mL, provides high specificity for the enrichment of phosphoproteins from complex biological samples. Concentrated Flow-through and Elution fractions were resolved on SDS-PAGE. Gel lanes were normalized by protein concentration, 10µg/lane. Western blot analysis was performed using phospho-specific antibodies. Cytochrome C is a negative control for nonspecific binding of non-phosphorylated proteins. High specificity of binding affinity for the phosphorylated target was demonstrated by the presence of Phospho-p42/44 MAPK in the Eluate and absence, thereof, in the Flow-through.

See page 53 for more information and other packaging formats of Pierce Protein Phosphoprotein Enrichment Resin.

Ordering Information

Product #	Description	Pkg. Size
87743	**Pierce Phosphoprotein Enrichment Chromatography Cartridges, 1mL** Formulation: 1mL resin cartridges with LC and Luer-Lok fittings. *Sufficient for: Purifying samples containing 4mg total protein (400µg phosphoprotein) per cartridge.*	2 cartridges
87744	**Pierce Phosphoprotein Enrichment Chromatography Cartridges, 5mL** Formulation: 5mL resin cartridge with LC and Luer-Lok fittings. *Sufficient for: Purifying samples containing 20mg total protein (2mg phosphoprotein) per cartridge.*	1 cartridge

Biotinylated Protein FPLC Purification

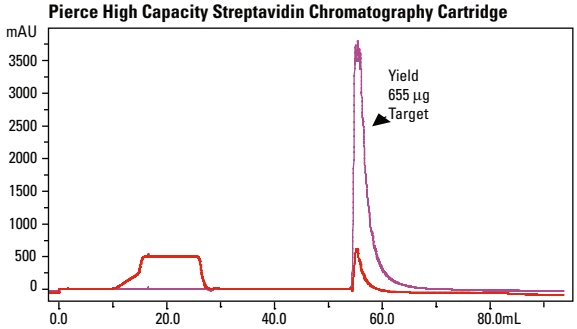
Thermo Scientific Streptavidin Agarose and Neutravidin Agarose Cartridges

Resins with high biotin affinity and low nonspecific binding.

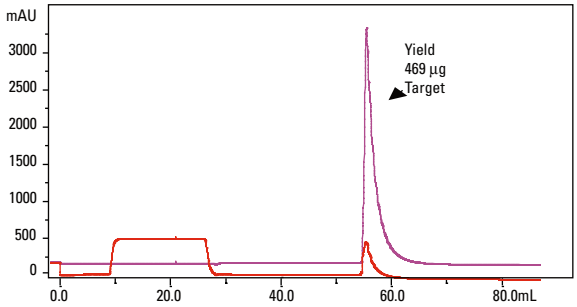
Highlights:

- Exhibit high binding affinity towards the vitamin Biotin (vitamin H)
- Captures biotinylated proteins
- Derivatives of the protein avidin from chicken egg whites that differ by their source and glycosylation level

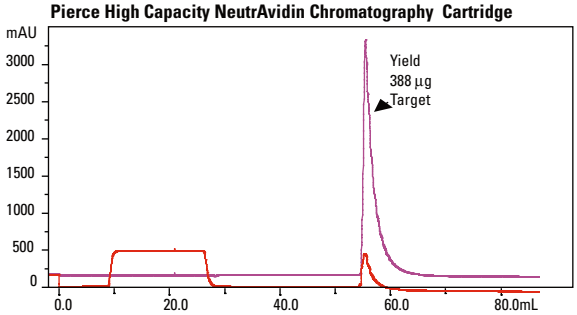
Pierce High Capacity Streptavidin Chromatography Cartridge



GE HiTrap Streptavidin HP



Pierce High Capacity NeutrAvidin Chromatography Cartridge



Thermo Scientific High Capacity Streptavidin Chromatography Cartridge achieves comparable yield and performance in binding of small biotinylated molecule, Biotin 4 Nitrophenyl ester (BpNPE), as compared to HiTrap.

Sample: 4.8mg BpNPE (in 24mL)
Column Size: 1mL
Binding Buffer: 0.5M Sodium Acetate, pH 5.0
Elution Buffer: 0.5M NaOH Streptavidin cartridges; 0.1M NaOH NeutrAvidin cartridge
Flow Rate: Sample Application 0.3mL/minute; Wash and Elution 1mL/minute
Monitored 2 wavelengths: Flow through/ Wash in Na Acetate A 270nm (red); Elution in NaOH A 410nm (pink)
Instrument: AKTApurifier
Yield determined by OD A 410nm (Extinction Coefficient for BpNPE 18.3 A410nm)

Supplier	Cartridge Size	Biotinylated BSA Bound
Pierce High Capacity Streptavidin Chromatography Cartridge	1mL	12.9mg
	5mL	75.9mg
GE HiTrap Streptavidin HP	1mL	10.7mg
	5mL	(Not offered in 5mL size)
Pierce High Capacity NeutrAvidin Chromatography Cartridge	1mL	12.8mg
	5mL	70.mg

Binding capacity of Thermo Scientific High Capacity Streptavidin Chromatography Cartridges is comparable to that of HiTrap. Columns were overloaded with Biotinylated BSA and purified per manufacturer's instructions. Binding capacity was determined using the Thermo Scientific Pierce BCA Protein Assay Kit (Product # 23225).

Note: *Capacity for the avidin resins was determined indirectly by subtracting the unbound biotinylated BSA present in the flow-through fractions from the total amount applied to the column.*

Comparison of biotin-binding proteins.

	Biotin-Binding Protein		
	Avidin	Streptavidin	NeutrAvidin
Molecular Weight	67K	53K	60K
Biotin-binding Sites	4	4	4
Isoelectric Point (pI)	10	6.8-7.5	6.3
Specificity	Low	High	Highest
Affinity for Biotin (K_d)	10^{-15}M	10^{-15}M	10^{-15}M
Nonspecific Binding	High	Low	Lowest

See pages 67-68 for more information and other packaging formats of Steptavidin and Neutravidin Agarose Resin

Ordering Information

Product #	Description	Pkg. Size
87739	**High Capacity Streptavidin Chromatography Cartridges, 1mL** Formulation: 1mL crosslinked 6% beaded agarose cartridges with LC and Luer-Lok fittings. *Sufficient for: Binding > 100µg biotin per cartridge (> 10mg biotinylated BSA per cartridge).*	2 cartridges
87740	**High Capacity Streptavidin Chromatography Cartridge, 5mL** Formulation: 5mL crosslinked 6% beaded agarose cartridge with LC and Luer-Lok fittings. *Sufficient for: Binding > 500µg biotin per cartridge (> 50mg biotinylated BSA per cartridge).*	1 cartridge
87741	**High Capacity NeutrAvidin Chromatography Cartridges, 1mL** Formulation: 1mL crosslinked 6% beaded agarose cartridges with LC and Luer-Lok fittings. *Sufficient for: Binding > 75µg biotin per cartridge (> 8mg biotinylated BSA per cartridge).*	2 cartridges
87742	**High Capacity NeutrAvidin Chromatography Cartridge, 5mL** Formulation: 5mL crosslinked 6% beaded agarose cartridge with LC and Luer-Lok fittings. *Sufficient for: Binding > 375µg biotin per cartridge (> 40mg biotinylated BSA per cartridge).*	1 cartridge

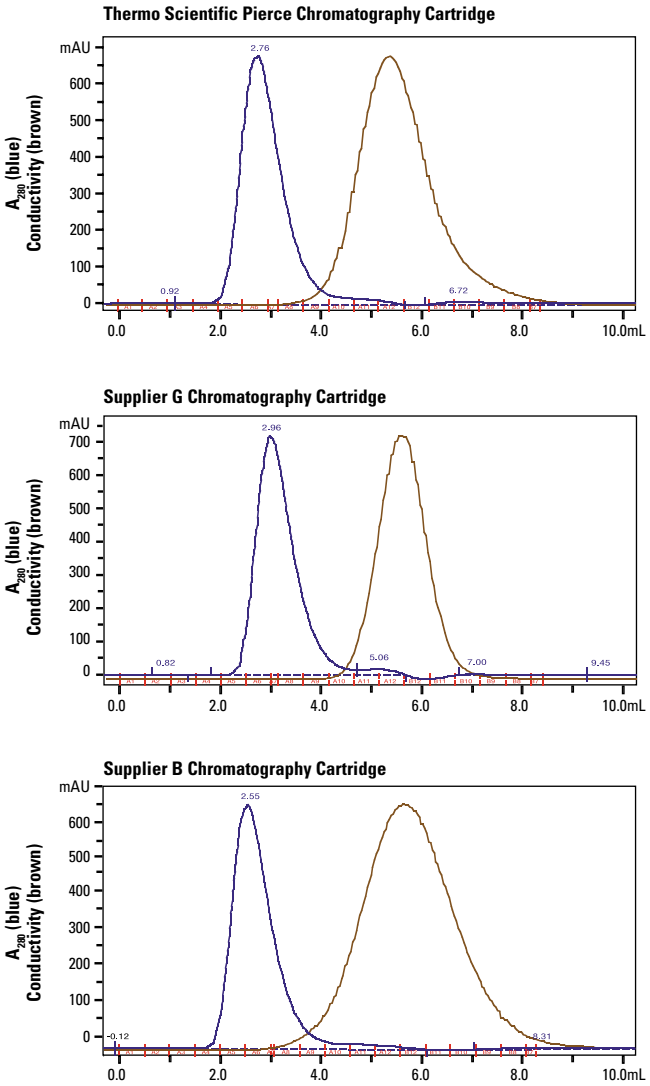
Protein Desalting

Thermo Scientific Zeba Desalting Chromatography Cartridges

The best protein desalting resin in cartridge format.

Highlights:

- Exceptional desalting and protein-recovery characteristics compared to other commercially available resins
- Successful with very dilute (25µg/mL) protein samples
- Greater than 95% retention (removal) of salts and other small molecules and good recovery of proteins and other macromolecules



Efficient salt removal and protein recovery with desalting chromatography cartridge. Bovine serum albumin (1mg) in 1M NaCl was applied to 5mL Thermo Scientific Zeba Desalting Cartridge (top) at a flow rate of 5mL/minute. Cartridge profile shows isocratic elution of BSA (blue) and NaCl detected by conductivity (brown). Greater than 95% of the BSA was recovered and more than 95% of the was salt removed. Results were comparable to those obtained with desalting cartridges from other suppliers (results for the Thermo Scientific Cartridge were essentially identical to that obtained with more expensive GE Healthcare and Bio-Rad Products (suppliers G and B, respectively).

Ordering Information

Product #	Description	Pkg. Size
89934	**Zeba Desalting Chromatography Cartridges, 7K MWCO, 1mL** Formulation: 1mL resin cartridges with LC and Luer-Lok fittings. *Sufficient for: Desalting 50 to 250µL samples per use.*	5 cartridges
89935	**Zeba Desalting Chromatography Cartridges, 7K MWCO, 5mL** Formulation: 5mL resin cartridges with LC and Luer-Lok fittings. *Sufficient for: Desalting 100 to 1500µL samples per use.*	5 cartridges

Additional Thermo Scientific Affinity Supports and Kits

In addition to the few affinity supports whose ligands have broad application to many different protein methods, there are many others whose applications are more narrowly defined or are incorporated into kits for very specific purposes. These include kits to isolate cell surface proteins using biotinylation and NeutrAvidin Agarose, kits to enrich for phosphoproteins, glycoproteins and ubiquitinated proteins. The stand-alone resins on this page and the next include those that can be used to remove trypsin from protein digest, isolate blood proteins, purify C-reactive protein and capture ribonucleosides. In addition, page 80 presents our complete line of magnetic beads, together with their magnet accessories.

Immobilized Soybean Trypsin Inhibitor

For effective removal of trypsin, chymotrypsin and elastase from protein digests.

Applications:

- Purifying trypsin, chymotrypsin and elastase[1,2]
- Removing proteases from activated pancreatic juices[3]

References

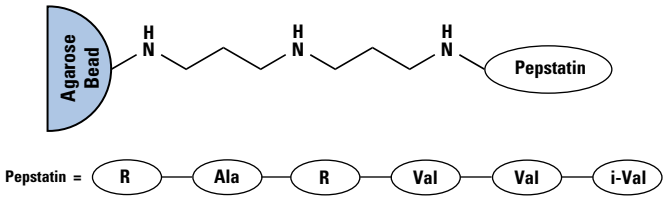
1. Feinstein, G., *et al.* (1974). *Euro. J. Biochem.* **43(3),** 569–581.
2. Peterson, L.M., *et al.* (1976). *Biochemistry* **15(12),** 2501–2508.
3. Reeck, G.R., *et al.* (1971). *Biochemistry* **10(25),** 4690–4698.

Ordering Information

Product #	Description	Pkg. Size
20235	**Immobilized Soybean Trypsin Inhibitor** Support: 4% beaded agarose Capacity: ≥6mg trypsin/mL of resin	2mL

Immobilized Pepstatin

An excellent cathepsin binding matrix.



R = (4-amino-3-hydroxy-6-methyl) heptanoic acid

Thermo Scientific Immobilized Pepstatin

Reference

Helseth, Jr., D.L. and Veis, A. (1984). *Proc. Natl. Acad. Sci. USA* **81,** 3302–3306.

Ordering Information

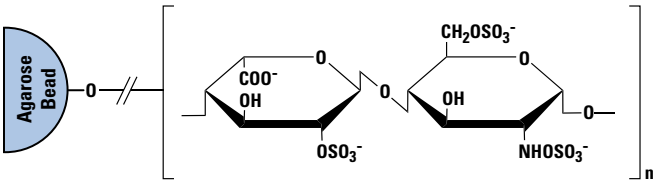
Product #	Description	Pkg. Size
20215	**Immobilized Pepstatin** Support: Crosslinked 6% beaded agarose Spacer: Diaminodipropylamine Capacity: 1–2mg of pepsin/mL of resin	5mL

Immobilized Heparin

Use to isolate many blood proteins that have enzymatic activities.

Applications:

- Enrich lysates for nucleic acid-binding proteins
- Isolate many blood proteins



Thermo Scientific Immobilized Heparin

Reference

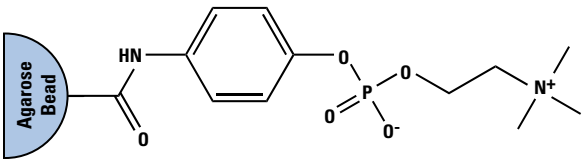
Smith, P.K., *et al.* (1980). *Anal. Biochem.* **109,** 466–473.

Ordering Information

Product #	Description	Pkg. Size
20207	**Immobilized Heparin** Support: 4% beaded agarose Loading: ≥0.2mg of heparin/mL of resin (determined by the colorimetric method)	Kit

Immobilized *p*-Aminophenyl Phosphoryl Choline

For C-reactive protein binding.



Thermo Scientific Immobilized *p*-Aminophenyl Phosphoryl Choline

Reference

Robey, F.A. and Liu, T.Y. (1981). *J. Biol. Chem.* **256,** 969–975.

Ordering Information

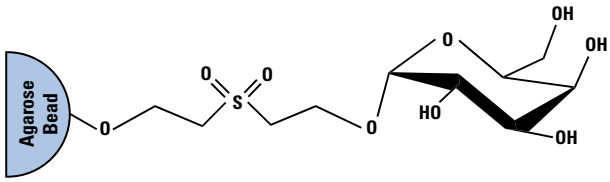
Product #	Description	Pkg. Size
20307	**Immobilized *p*-Aminophenyl Phosphoryl Choline** Support: Crosslinked 6% beaded agarose Capacity: ≥3mg of human C-reactive protein/mL of resin	5mL

Immobilized D-Galactose

For lectin and galactosidase binding.

Applications:

- Human alpha-galactosidase A purification[1]
- *E. coli* heat labile enterotoxin purification[2]
- C-type lectin purification[3]
- Cholera toxin (CT) purification[4,5]



Thermo Scientific Immobilized Thio-alpha-D-Galactose

References

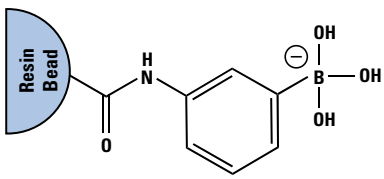
1. Yasuda, K., *et al.* (2004). *Protein Expression and Purification.* **37,** 499–506.
2. Okamoto, K., *et al.* (1998). *J. Bacteriol.* **180,** 1368–1374.
3. Matsumoto, J., *et al.* (2001). *Development.* **128,** 3339–3347.
4. Bowman, C.C. and Clements, J.D., *et al.* (2001). *Infec. Immun.* **69,** 1528–1535.
5. Tinker, J.K., *et al.* (2003). *Infec. Immun.* **71,** 4093–4101.

Ordering Information

Product #	Description	Pkg. Size
20372	**Immobilized D-Galactose** Support: 6% beaded agarose Capacity: ≥20mg jacalin/mL resin	5mL

Immobilized Boronic Acid

For ribonucleoside isolation.



Thermo Scientific Immobilized Boronic Acid

References

Vlassara, H., *et al.* (1981). *Proc. Natl. Acad. Sci. USA* **78,** 5190–5192.
Gehrke, C.W., *et al.* (1978). *J. Chromatogr.* **150,** 455–476.

Ordering Information

Product #	Description	Pkg. Size
20244	**Immobilized Boronic Acid** Support: Polyacrylamide resin beads Capacity: ≥99% binding and recovery of 110µmol AMP/mL of resin Spacer: *m*-aminophenyl group Loading: 100µmol boronate/mL of resin	10mL

Thermo Scientific MagnaBind Beads and Supports

A convenient method for isolating biomolecules using affinity binding, while retaining biological activity.

Magnetic beads are a convenient affinity support for a variety of assays, which allow easy purification of the target without columns or centrifugation. After a binding step in an affinity purification procedure, the magnetic particles are easily and rapidly collected by placing the microcentrifuge tube or reaction vessel next an appropriate rare-earth magnet (Figure 1). Thermo Scientific MagnaBind beads respond rapidly to MagnaBind Magnets but can be easily dispersed and regathered multiple times (i.e., they will not irreversibly aggregate) because they do not have any magnetic memory. MagnaBind Beads are available pre-coated with Protein A, Protein G, streptavidin, anti-mouse or anti-rabbit antibodies. Activated beads, with either amine or carboxyl groups, are also available for attaching other proteins or affinity ligands to a magnetic particle.

Highlights:

- Available pre-coated with popular affinity ligands or derivatized for covalent attachment of proteins and other specific ligands
- Beads do not irreversibly aggregate because they have no magnetic memory; collect and disperse the beads multiple times if needed
- Most separations require a short five- to 10-minute bench-top procedure

Applications:

- Cell sorting using positive or negative selection
- Protein purification or immunoassays using direct or indirect methods

Characteristics of underivatized Thermo Scientific MagnaBind Beads.

Composition	Silanized iron oxide
Magnetization	25–35EMU/g
Type of Magnetization	Superparamagnetic (no magnetic memory)
Surface Area	>100m^2/g
Settling Rate	4% in 30 minutes
Effective Density	2.5g/mL
Number of Beads	1 x 10^8 beads/mg
pH Stability	Aqueous solution, above pH 4.0
Concentration	5mg/mL

Note: *To establish a microbe-free preparation, MagnaBind Beads can be washed with antibiotic medium or γ-irradiated.*

Figure 1. Thermo Scientific MagnaBind Magnet for 1.5mL Microcentrifuge Tube. Thermo Scientific MagnaBind Beads in solutions within a microcentrifuge tube are rapidly "pelleted" when the tube is placed in the magnetized holder. Magnets for six microcentrifuge tubes and 96-well microplates are also available.

References
Chaudhuri, T.K., *et al.* (2001). *Cell* **107,** 235–246.
Newey, S.E., *et al.* (2001). *J. Biol. Chem.* **276,** 6645–6655.
Xu, X., *et al.* (2001). *J. Biol. Chem.* **276,** 43221–43230.

Ordering Information

Product #	Description	Pkg. Size
21344	MagnaBind Streptavidin Beads	5mL
21348	MagnaBind Protein A Beads	5mL
21349	MagnaBind Protein G Beads	5mL
21354	MagnaBind Goat Anti-Mouse IgG Beads	50mL
21356	MagnaBind Goat Anti-Rabbit IgG Beads	50mL
21353	MagnaBind Carboxyl Derivatized Beads	5mL
21352	MagnaBind Amine Derivatized Beads	5mL
21358	MagnaBind Magnet for 96-Well Plate Separator	1 magnet
21357	MagnaBind Magnet for 1.5mL Microcentrifuge Tube	1 magnet
21359	MagnaBind Magnet for 6 x 1.5mL Microcentrifuge Tubes	1 magnet

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