

# New magnetic beads support Thermo Scientific KingFisher Instruments

Fast protocols for manual and automated processing

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We now offer magnetic beads for use with the Thermo Scientific KingFisher Flex and KingFisher 96 Automated Platforms for magnetic isolation/purification of proteins. The instruments support large-scale screening of multiple samples in proteomics workflows. Our new magnetic beads, including Protein A, Protein G, glutathione and titanium dioxide, are fully compatible with the KingFisher Instruments. The Thermo Scientific Pierce Protein A Magnetic Beads and Protein G Magnetic Beads are typically used for isolating antibodies from serum, cell culture supernatant or ascites and for immunoprecipitating antigens from cell or tissue extracts. The Thermo Scientific Pierce Glutathione Magnetic Beads purify glutathione S-transferase (GST)-fusion proteins from crude cell lysate prepared from bacteria, yeast or mammalian cells. The Thermo Scientific Pierce Magnetic TiO<sub>2</sub> Phosphopeptide Enrichment Kit is for isolating phosphopeptides from complex biological samples using titanium dioxide-coated magnetic beads. The isolated phosphopeptides are analyzed downstream by mass spectrometry. Fast and convenient protocols are available for both manual and automated processing using each of the magnetic beads (Table 1).

## Methods

Note: All experiments were performed on the KingFisher 96 Instrument (Product No. 5400500) using Deep Well 96 Plates (Product No. 95040450) and Thermo Scientific BindIt Software. Referenced protocols were downloaded from the Thermo Scientific web site ([www.thermo.com/kingfisher](http://www.thermo.com/kingfisher)) on an external computer and subsequently transferred to the instrument.

**Serum IgG purification:** Approximately 0.5 mg of Pierce Protein A Magnetic Beads were added to 16 wells of a Deep Well 96 Plate. IgG was purified from rabbit serum using the "Antibody Purification" protocol. Briefly, beads were washed in Tris-buffered saline (TBS) containing 0.1% Tween-20 (TBST), incubated 1 hour with 5 mg rabbit serum per well, washed in TBST and eluted in 0.1 M glycine, pH 2.8. Eluates were resolved by SDS-PAGE and stained with Thermo Scientific Imperial Protein Stain (Product No. 24615).

**Immunoprecipitation (IP) of Grp94:** MOPC cell lysate (0.75 µg per sample) was combined with and without anti-Grp94 antibody (10 µg) and incubated overnight at 4°C. Isolation of the Grp94/antibody pair was performed on the KingFisher 96 Instrument using the "Immunoprecipitation Heated Elution" protocol. Briefly, Pierce Protein G Magnetic Beads (0.5 mg or 0.75 mg per well) were added to a Deep Well 96 Plate and washed with TBST. The antigen sample/antibody mixture was incubated for 1 hour with the beads. The beads were washed and eluted for 10 minutes at 96°C with SDS-PAGE reducing sample buffer. Alternatively, the same procedure was performed manually using a magnetic stand and microcentrifuge tubes. Eluates were resolved by SDS-PAGE and stained with Imperial\* Protein Stain. The Grp94 gel band was excised from the gel, digested with trypsin and analyzed on a Thermo Scientific LTQ XL Mass Spectrometer.

**GST-Rabaptin Purification:** Pierce Glutathione Magnetic Beads, 100 µl per well were added to 13 wells of a Deep Well 96 Plate with Binding/Wash Buffer (125 mM Tris, 150mM NaCl, pH 8.0) to a final volume of 200 µl. Protein purification was performed using the "GST Protein Purification" protocol. Briefly, beads were washed in Binding/Wash Buffer and incubated 1 hour with bacterial cell lysate. The beads were washed and eluted with 50 mM reduced glutathione prepared in Binding/Wash Buffer. Eluates were resolved by SDS-PAGE and stained with Imperial Protein Stain.

**Table 1. Applications for Thermo Scientific Pierce Magnetic Beads.**

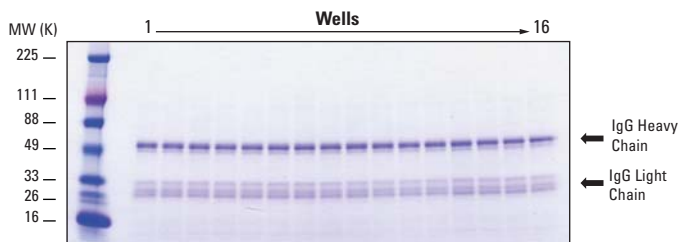
Thermo Scientific Pierce Magnetic Bead	Application and Sample Source	Recommended Detection Methods
Protein A	<ul style="list-style-type: none"> <li>Purify antibodies from serum, cell culture supernatant or ascites</li> <li>Immunoprecipitate antigens from cell or tissue extracts</li> </ul>	<ul style="list-style-type: none"> <li>SDS-PAGE gels</li> <li>Western blot</li> </ul>
Protein G	<ul style="list-style-type: none"> <li>Purify antibodies from serum, cell culture supernatant or ascites</li> <li>Immunoprecipitate antigens from cell or tissue extracts</li> </ul>	<ul style="list-style-type: none"> <li>SDS-PAGE</li> <li>Western blot</li> <li>Mass spectrometry</li> </ul>
Glutathione	<ul style="list-style-type: none"> <li>Purify GST-fusion proteins from crude cell lysate prepared from bacteria, yeast or plant or mammalian cells</li> </ul>	<ul style="list-style-type: none"> <li>SDS-PAGE</li> <li>Western Blot</li> <li>Mass Spectrometry</li> </ul>
Titanium Dioxide (Kit)	<ul style="list-style-type: none"> <li>Isolate and enrich phosphopeptides from complex biological samples</li> </ul>	<ul style="list-style-type: none"> <li>Mass spectrometry</li> </ul>

# Magnetic Beads for Automated Solutions

**Phosphopeptide Enrichment from PBMCs:** A tryptic digest of lymphocytes was processed using the Pierce Magnetic Titanium Dioxide Phosphopeptide Enrichment Kit. Samples were prepared using the “Phosphopeptide Enrichment-Deep Well” protocol on a KingFisher 96 Instrument. Briefly, 100  $\mu$ l of TiO<sub>2</sub> magnetic beads were added to a Deep Well 96 Plate, rinsed with Binding Buffer and bound to 2 mg of peptide digest per well. The beads were washed and eluted. Eluted samples were dried, rehydrated in 5% acetonitrile/1% formic acid in water and injected into a Thermo Scientific LTQ-FT ultra high-resolution mass spectrometer.

## Results and Discussion

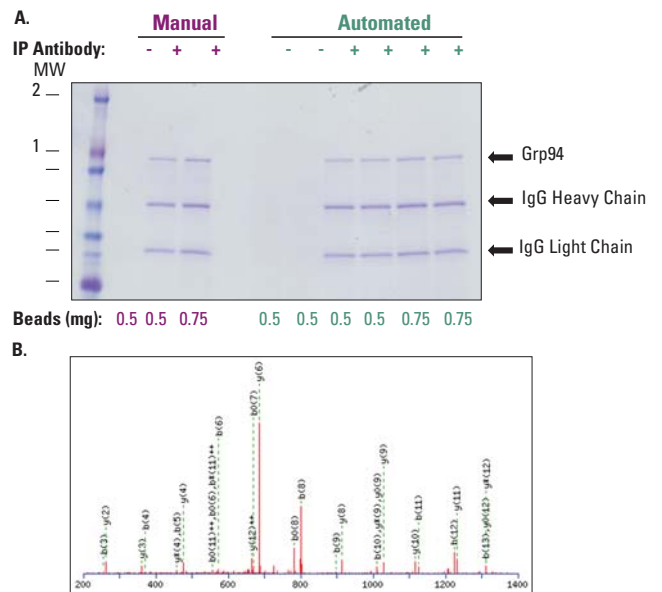
Pierce Magnetic Beads were tested in a variety of applications to demonstrate their utility in proteomics workflows using the KingFisher 96 Instrument. Rabbit serum IgG was purified using Pierce Protein A Magnetic Beads with excellent reproducibility across 16 wells (Figure 1). Samples were also processed in an additional 32 wells with similar results (data not shown).



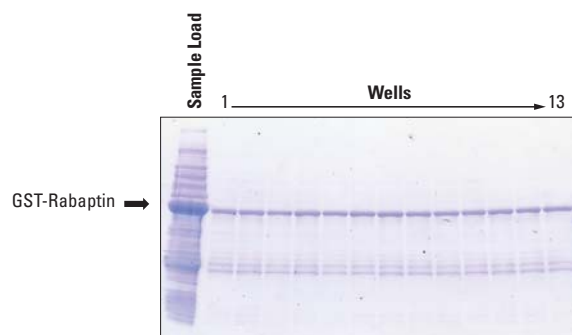
**Figure 1. Serum IgG purification with Thermo Scientific Pierce Protein A Magnetic Beads.** IgG was purified from 5 mg of rabbit serum in 16 wells of a 96-well plate on a KingFisher 96 Instrument using 0.5 mg of magnetic beads per well. The diluted samples were resolved by SDS-PAGE and stained with Thermo Scientific Imperial Protein Stain.

Grp94 immunoprecipitation from MOPC cell lysate was performed with Pierce Protein G Magnetic Beads. Both manual and automated protocols produced similar results (Figure 2A). The beads exhibited low nonspecific binding, as demonstrated by the absence of protein bands in addition to Grp94 and IgG bands in the gel. The Grp94 band was excised from the gel, digested and analyzed by mass spectrometry. The protein was identified with 39% sequence (Figure 2B). GST-Rabaptin was purified from bacterial cell lysate using Pierce Glutathione Magnetic Beads. GST-Rabaptin was isolated reproducibly across 13 wells (Figure 3). Mass spectrometry analysis of phosphopeptides enriched with the Pierce TiO<sub>2</sub> Phosphopeptide Enrichment Kit revealed 177 unique phosphopeptides, compared with only one isolated in a nonenriched sample (Figure 4 and Table 2).

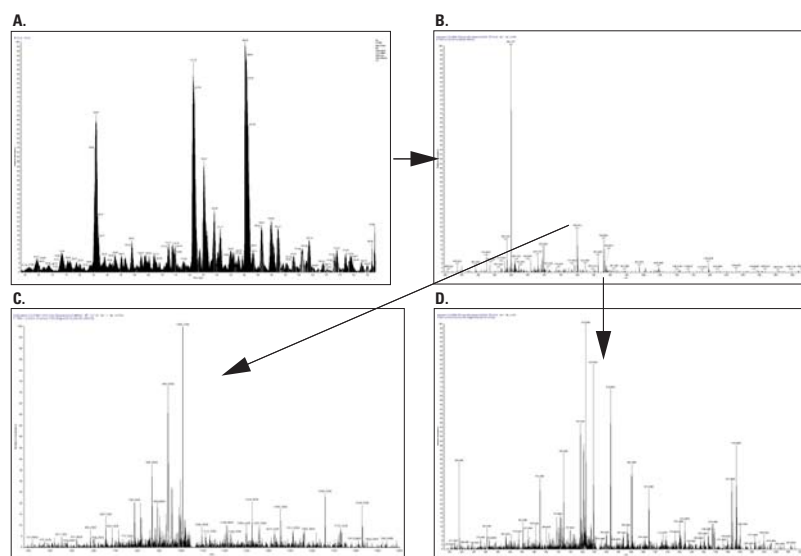
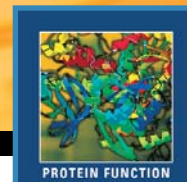
Overall, automation with Pierce Magnetic Beads on a KingFisher 96 Instrument produced high-quality, reproducible data in a fast and convenient format.



**Figure 2. Manual vs. automated Grp94 immunoprecipitation.** Panel A: Grp94 was isolated from MOPC cells using manual and automated protocols. Eluates prepared from 0.5 and 0.75 mg of Pierce Protein G Magnetic Beads were analyzed by SDS-PAGE. The negative controls (no IP antibody) were prepared with 0.5 mg of bead. Panel B: MS/MS spectrum of a representative peptide (GVVDSDDLPLNVSRR) of Grp94 identified from a band excised from the gel in Panel A. Digested protein was analyzed with an LTQ XL Mass Spectrometer and Grp94 was identified with 39% sequence.



**Figure 3. Purification of GST-Rabaptin using glutathione beads.** GST-Rabaptin was purified from bacterial lysate using 100  $\mu$ l of Pierce Glutathione Magnetic Beads on a KingFisher 96 Instrument. All eluates were normalized by volume and analyzed by SDS-PAGE.



**Figure 4.** High-resolution LC-MS/MS data obtained from 2 mg of a tryptic peptide digest prepared from peripheral blood mononuclear cells (lymphocytes) enriched with the Thermo Scientific Pierce TiO<sub>2</sub> Phosphopeptide Enrichment Kit. Peptide samples were processed on a KingFisher 96 Instrument. **Panel A.** Full scan chromatogram obtained on an LTQ-FT Ultra High-Resolution Mass Spectrometer, resolution 200K, mass accuracy < 2 ppm. **Panel B.** Zoom of one full scan, retention

time 64.50 minutes. **Panel C.** MS<sup>2</sup> fragmentation spectrum of single phosphopeptide ion, parent mass 1582.70. Peptide sequence: SS' PFKVS' PLTFGR. The protein was identified as serum deprivation response protein. **Panel D.** MS<sup>2</sup> fragmentation spectrum of a single phosphopeptide ion, parent mass 1722.80. The peptide sequence is LPS' GSGAASPTGSAVDIR. The protein was identified as AHNAK nucleoprotein isoform 1. ( ' = site of phosphorylation).

**Table 2. Summary of the Figure 4 data obtained from sample enriched and not enriched with the Thermo Scientific Pierce TiO<sub>2</sub> Phosphopeptide Enrichment Kit.**

	Enriched	Non-Enriched
Total number of proteins identified	185	247
Total number of phosphoproteins identified	160	1
Total number of peptides identified	28347	28457
Total number of phosphopeptides identified	28009	7
Total number of unique phosphopeptides identified	177	1
Relative enrichment for phosphopeptides (%)	86	0.3

## Thermo Scientific Pierce Protein Reagents

### Ordering Information

Product #	Description	Pkg. Size
88800	<b>Protein A Magnetic Beads</b>	1 ml
88801	<b>Protein A Magnetic Beads</b>	5 ml
88806	<b>Protein G Magnetic Beads</b>	1 ml
88807	<b>Protein G Magnetic Beads</b>	5 ml
88821	<b>Glutathione Magnetic Beads</b>	4 ml
88822	<b>Glutathione Magnetic Beads</b>	20 ml
88811	<b>Magnetic Titanium Dioxide Phosphopeptide Enrichment Kit, Trial Size</b> Includes: Magnetic TiO <sub>2</sub> Beads, 20X Binding Buffer Washing Buffer Elution Buffer Thermo-Fast 96 Robotic PCR Plate, 0.2 ml well volume	Kit 20X, 1 ml 100 ml 25 ml 3 ml 2 ea.
88812	<b>Magnetic Titanium Dioxide Phosphopeptide Enrichment Kit, Trial Size</b> Includes: Magnetic TiO <sub>2</sub> Beads, 20X Binding Buffer Washing Buffer Elution Buffer Thermo-Fast 96 Robotic PCR Plate, 0.2 ml well volume	Kit 0.25 ml 100 ml 25 ml 3 ml 2 ea.
24615	<b>Imperial Protein Stain</b>	1 L
34095	<b>SuperSignal West Femto Maximum Sensitivity Substrate<sup>†</sup></b>	100 ml
AB1300	<b>Thermo-Fast 96 Robotic PCR Plate, 0.2 ml well volume<sup>†</sup></b>	50 plates per box

## Thermo Scientific KingFisher Flex Products

### Ordering Information

Product #	Description	Pkg. Size
5400610	<b>KingFisher Flex 96 PCR head</b>	1 ea.
5400620	<b>KingFisher Flex 96 KF head</b>	1 ea.
5400630	<b>KingFisher Flex 96 deep well head</b>	1 ea.
5400640	<b>KingFisher Flex 24 deep well head</b>	1 ea.
97002514	<b>KingFisher Flex 96 tip comb for PCR magnets for PCR magnets</b>	80 pcs.
97002524	<b>KingFisher Flex 96 tip comb for KF 96 magnets</b>	100 pcs.
97002534	<b>KingFisher Flex 96 tip comb for DW magnets</b>	100 pcs.
97002610	<b>KingFisher Flex 24 deep well tip comb and plate</b>	50 pcs. of each
97002540	<b>KingFisher 96 KF plate (200 µl)</b>	48 pcs.
95040450	<b>Microtiter Deepwell 96 Plate, V-bottom</b>	50 pcs.
95040460	<b>Microtiter Deepwell 96 Plate, V-bottom Sterile</b>	50 pcs.
95040470	<b>KingFisher Flex 24 Deep Well Plate</b>	50 pcs.
95040480	<b>KingFisher Flex 24 Deep Well Plate Sterile</b>	50 pcs.

\* Trademark, see Trademark Index on page 35.

† Patent, see patent info on page 35.

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