

# NAb™ Spin Columns, 0.2 ml for Antibody Purification

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<b>Number</b>	<b>Description</b>
<b>89952</b>	<b>NAb Protein A Plus Spin Columns, 0.2 ml</b> , 10 each Binding Capacity: ≥ 7.0 mg human IgG per column
<b>89953</b>	<b>NAb Protein G Spin Columns, 0.2 ml</b> , 10 each Binding Capacity: 2.2-3.0 mg human IgG per column
<b>89954</b>	<b>NAb Protein A/G Spin Columns, 0.2 ml</b> , 10 each Binding Capacity: ≥ 1.4 mg human IgG per column
<b>89955</b>	<b>NAb Protein L Spin Columns, 0.2 ml</b> , 10 each Binding Capacity: 1.0-2.0 mg human IgG per column

Contents: Columns are supplied with top and bottom caps. Each column contains a 0.2 ml resin bed of crosslinked 6% beaded agarose in 0.02% sodium azide.

**Storage:** Upon receipt store columns at 4°C. Columns are shipped at ambient temperature.

## Introduction

NAb Spin Columns are convenient for rapid, small-scale affinity purification of antibodies from a variety of sample types. Each column containing 0.2 ml of the immobilized protein resin enables quick purification of 100-1,000 µg of IgG from 25-500 µl of serum or other sample. The actual amount of IgG purified varies depending upon the sample type and the specific spin column used.

Proteins A, G and L are different bacterial proteins that bind with high specificity to mammalian immunoglobulins. Immobilized forms of these proteins have been widely used for affinity purification of antibodies from serum, ascites fluid and hybridoma culture supernatant samples. The particular species and class of antibody to be purified determines which one of these immobilized protein resins is most appropriate. The following paragraphs provide general guidelines for making this choice; consult the Pierce catalog or website for a more detailed description and table of antibody-binding characteristics for Proteins A, G, A/G and L.

Proteins A and G bind to many of the same species and subclasses of IgG, although they have particular differences in affinity and binding capacity. Protein A is generally preferred for affinity purification of rabbit, pig, dog and cat IgG. Protein G has better binding capacity for a broader range of mouse and human IgG subclasses (IgG<sub>1</sub>, IgG<sub>2</sub>, etc.). Protein A/G is a recombinant fusion protein that includes the IgG-binding domains of both Protein A and Protein G. Therefore, Protein A/G is ideal for binding the broadest range of IgG subclasses from rabbit, mouse, human and other mammalian samples.

Protein L binds to certain immunoglobulin kappa light chains. Because kappa light chains occur in members of all classes of immunoglobulin (i.e., IgG, IgM, IgA, IgE and IgD), Protein L can purify these different classes of antibody. However, only those antibodies within each class that possess the appropriate kappa light chains will bind. Generally, empirical testing is required to determine if Protein L is effective for purifying a particular antibody of interest.

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## Additional Materials Required

- 2 ml microcentrifuge tubes (Product No. 69720)
- Microcentrifuge set to  $5,000 \times g$  for all centrifugation steps
- Binding Buffer: 0.1 M phosphate, 0.15 M sodium chloride; pH 7.2 (BupH™ Phosphate Buffered Saline Packs, Product No. 28372) – alternatively, use a buffer optimized for the specific antibody-binding protein, such as one of the following buffers:
  - Protein A IgG Binding Buffer (Product No. 21001 or 21007)
  - Protein G IgG Binding Buffer (Product No. 21011)
  - Protein A/G IgG Binding Buffer (Product No. 54200)
- Elution Buffer: IgG Elution Buffer (Product No. 21004 or 21009) or 0.1 M glycine, pH 2-3
- Neutralization Buffer: 2 ml of high-ionic strength alkaline buffer such as 1 M phosphate or 1 M Tris at pH 8-9
- Storage Solution: 0.02% sodium azide in phosphate-buffered saline

## Procedure for Antibody Purification

**Note:** Typically, the immobilized protein column may be used up to 10 times without significant loss in binding capacity.

1. Equilibrate column and buffers to room temperature. Set microcentrifuge to  $5,000 \times g$ .
2. Snap off bottom closure and loosen cap on spin column. Place column in a 2 ml collection tube, centrifuge for 1 minute and discard the flow-through.
3. Equilibrate column by adding 400  $\mu$ l of Binding Buffer to the column/collection tube assembly and mix briefly. Centrifuge the column and discard the flow-through. Repeat this step once.
4. Cap bottom of spin column with the included rubber cap, add 25-500  $\mu$ l of antibody-containing sample and cap the column top.
5. Incubate column at room temperature with end-over-end mixing for 10 minutes, when volumes allow mixing to occur.
6. Loosen cap and remove bottom cap. Place spin column in new collection tube and centrifuge for 1 minute.

**Note:** This first collection tube contains the nonbound sample components and can be analyzed to assess binding efficiency and capacity.

7. Transfer the column to a new collection tube. Wash column by adding 400  $\mu$ l of Binding Buffer. Mix briefly to suspend the resin and centrifuge for 1 minute. Repeat wash two additional times for a total of three washes.
8. Add 40  $\mu$ l of Neutralization Buffer to three collection tubes and place the spin column into one of the tubes.
9. Add 400  $\mu$ l of IgG Elution Buffer to the spin column, mix gently and centrifuge for 1 minute. Transfer the spin column to another collection tube that contains Neutralization Buffer, saving the collected solution as the first elution fraction. Repeat this step two times to obtain three fractions.
10. Determine which fraction(s) contain the purified antibody by measuring the relative absorbance of each fraction at 280 nm. If required for downstream applications, exchange the buffer using Zeba™ Desalt Spin Columns, or by using Slide-A-Lyzer® Dialysis Cassettes or Slide-A-Lyzer MINI Dialysis Units (see Related Products section).
11. To regenerate the used column for storage or re-use, add 400  $\mu$ l of Elution Buffer and centrifuge for 1 minute. Repeat three times. Wash resin several times with Storage Solution and store column at 4°C. Do not allow the resin to become dry. Typically, the immobilized protein column may be used up to 10 times without significant loss in binding capacity, although the actual number of effective usages may vary.

## Troubleshooting

Problem	Possible Cause	Solution
No protein detected in any elution fractions by absorbance at 280 nm or general protein staining of electrophoresed sample	Sample devoid of any antibody species or isotype that binds to the immobilized protein used (e.g., no antibodies in sample contain kappa light chains when using Immobilized Protein L)	Ensure by other means, such as an ELISA or isotyping kit, that the sample contains IgG-type antibody (see Related Products)
Considerable antibody purified, but no specific antibody of interest detected	Antibody of interest is at low concentration or has low binding affinity for the immobilized protein relative to other immunoglobulins in the sample	Use serum-free medium for cell supernatant samples
		Affinity-purify the antibody using the specific antigen coupled to a support such as AminoLink® Plus Kit (see Related Products)
Antibody of interest purified, but it is denatured (as determined by lack of function in downstream assay)	Antibody is sensitive to low-pH Elution Buffer	Try Gentle Ag/Ab Elution Buffer (see Related Products)
	Downstream application is sensitive to neutralized Elution Buffer	Desalt or dialyze eluted sample into an application-compatible buffer

## Additional Information

Please visit the web site for additional information relating to this product including the following items:

- Tech Tip # 34: Binding characteristics for immunoglobulin proteins and Proteins A, G, A/G and L
- Tech Tip # 43: Protein stability and storage

## Related Products

89868	Pierce Spin Columns, 50 units
69720	Pierce Microcentrifuge Tubes, 2 ml, 72 tubes
21027	Gentle Ag/Ab Elution Buffer, 500 ml
37501	Monoclonal Antibody Isotyping Kit I (HRP/ABTS)
44894	AminoLink Plus Immobilization Kit
89882	Zeba Desalt Spin Columns, 0.5 ml, 25/pkg, sample volumes of 30-130 µl
89889	Zeba Desalt Spin Columns, 2 ml, 5/pkg sample volumes 200-700 µl
66385	Slide-A-Lyzer Dialysis Cassette Kit, 10 dialysis cassettes, each appropriate for 0.1-0.5 ml samples
69576	Slide-A-Lyzer MINI Dialysis Units Plus Float, 10 units, each appropriate for 10-100 µl samples

Slide-A-Lyzer® MINI Dialysis Unit Technology is protected by U.S. Patent # 6,039,871.

Slide-A-Lyzer® Dialysis Cassette Technology is protected by U.S. Patent # 5,503,741 and 7,056,440.

This product ("Product") is warranted to operate or perform substantially in conformance with published Product specifications in effect at the time of sale, as set forth in the Product documentation, specifications and/or accompanying package inserts ("Documentation") and to be free from defects in material and workmanship. Unless otherwise expressly authorized in writing, Products are supplied for research use only. No claim of suitability for use in applications regulated by FDA is made. The warranty provided herein is valid only when used by properly trained individuals. Unless otherwise stated in the Documentation, this warranty is limited to one year from date of shipment when the Product is subjected to normal, proper and intended usage. This warranty does not extend to anyone other than the original purchaser of the Product ("Buyer").

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Current versions of product instructions are available at [www.thermo.com/pierce](http://www.thermo.com/pierce). For a faxed copy, call 800-874-3723 or contact your local distributor.

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