INSTRUCTIONS

NAb™ Spin Columns, 1mL

For Antibody Purification

89956 89957 89958 89959 1940.2

Number	Description
89956	**NAb Protein A Plus Spin Column, 1mL,** 5 each Binding Capacity**:** ≥ 34mg human IgG per column
89957	**NAb Protein G Spin Column, 1mL,** 5 each Binding Capacity 11-15mg human IgG per column
89958	**NAb Protein A/G Spin Column, 1mL,** 5 each Binding Capacity: ≥ 7mg human IgG per column
89959	**NAb Protein L Spin Column, 1mL,** 5 each Binding Capacity: 5-10mg human IgG per column

Contents: Columns are supplied with top and bottom caps. Each column contains a 1mL 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

The Thermo Scientific NAb Spin Columns are convenient for fast, small-scale affinity purification of antibodies from a variety of sample types. Each column containing 1mL of the immobilized protein resin enables quick purification of 1-13mg of IgG from 0.5-2mL 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 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_1, IgG_2, 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.

Additional Materials Required

- 15mL collection tubes
- Centrifuge set to 1,000 × *g* for all centrifuge steps
- Binding Buffer: 0.1M phosphate, 0.15M sodium chloride; pH 7.2 (PBS; Thermo Scientific 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 0.1M glycine, pH 2-3
- Neutralization Buffer: 2mL of high-ionic strength alkaline buffer such as 1M phosphate or 1M Tris at pH 8-9
- Storage Solution: 0.02% sodium azide in PBS

Spin Purification Protocol

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 centrifuge to 1,000 × *g*.
2. Prepare sample for purification by diluting to 2mL in Binding Buffer (maximum volume).
3. Loosen top cap on spin column and snap off bottom closure. Place column in a 15mL collection tube and centrifuge for 1 minute to remove storage solution. Discard the flow-through.
4. Equilibrate column by adding 2mL of Binding Buffer. Centrifuge for 1 minute and discard the flow-through. Repeat.
5. Cap bottom of column with the included rubber cap. Apply sample to column and tightly cap to top. Incubate at room temperature with end-over-end mixing for 10 minutes.
6. Loosen top cap and remove bottom cap. Place column in a new 15mL collection tube and centrifuge for 1 minute. Save the flow-through.

   Note: This first collection tube contains the nonbound sample components and can be analyzed to assess binding efficiency and capacity.
7. Transfer column to a new 15mL collection tube. Wash column by adding 2mL of Binding Buffer and centrifuging for 1 minute. Repeat for a total of three washes.
8. Add 100 µl of Neutralization Buffer to three 15mL collection tubes and place the spin column into one of the tubes.
9. Add 1mL Elution Buffer to the column and centrifuge for 1 minute into the first of the three collection tubes with Neutralization Buffer. 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 280nm. If required for downstream applications, exchange the buffer using Thermo Scientific Zeba Desalt Spin Columns or Thermo Scientific Slide-A-Lyzer Dialysis Cassettes (see related Thermo Scientific Products section).
11. To regenerate the column for storage or re-use, add 3mL of Elution Buffer and centrifuge for 1 minute. Repeat. Wash column with 3mL of PBS to remove elution buffer. Add 3mL of Storage Solution and store column at 4°C. 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.

Gravity-flow Purification Protocol

1. Equilibrate column and buffers to room temperature.
2. Dilute sample 1:1 with Binding Buffer.
3. Gently tap column on bench top to dislodge any resin that may be in the top cap. Gently snap off bottom closure and remove top cap. Place column in a 15mL collection tube and allow storage solution to drain.
4. Equilibrate column by adding 5mL of Binding Buffer and allow the solution to drain.
5. Apply the diluted sample to the column. For best results, add a sample volume that is less than 80% of the column's antibody-binding capacity. Collect the flow-through.

   Note: If the sample contains more IgG than can bind to the column, the flow-through will contain the excess antibody. By saving the flow-through, nonbound antibody can be recovered and analyzed.
6. Wash column with 15mL of Binding Buffer.

   Note: If desired, verify that all nonbound proteins are removed from the column by collecting separate 2mL fractions as the column drains. Measure the absorbance of each fraction at 280 nm. The last fractions should have an absorbance similar to the Binding Buffer.
7. Add 100 µl Neutralization Buffer to five collection tubes. Elute antibodies with 5mL of Elution Buffer, collecting 1mL fractions in each of the buffer-containing tubes.
8. Measure the relative absorbance of each fraction at 280 nm and pool fractions as desired. If required for downstream applications, exchange the buffer using Zeba Desalt Spin Columns or by using Slide-A-Lyzer Dialysis Cassettes (see related Thermo Scientific Products section).
9. Regenerate column by adding 8mL of Elution Buffer and allow solution to flow through the column.
10. Store column by adding 5mL of Storage Solution. When approximately 3mL remain in the column, cap bottom and secure top cap. Store column at 4°C.

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 Thermo Scientific 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 (see Related Thermo Scientific 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 the Gentle Ag/Ab Elution Buffer (see Related Thermo Scientific Products)
	Downstream application is sensitive to neutralized Elution Buffer	Desalt or dialyze eluted sample into an application-compatible buffer

Additional Information

Please visit our website for additional information relating to this product including the following items:

- Tech Tip: Binding Characteristics for Immunoglobulin Proteins and Proteins L, A, G, and A/G
- Tech Tip: Protein Stability and Storage

Related Thermo Scientific Products

21001	**Protein A IgG Binding Buffer,** 1L
21011	**Protein G IgG Binding Buffer,** 3.75L
54200	**Protein A/G IgG Binding Buffer,** 240mL
21027	**Gentle Ag/Ab Elution Buffer**, 500mL
37501	**Monoclonal Antibody Isotyping Kit I (HRP/ABTS)**
28372	**BupH Phosphate Buffered Saline Packs**, 40/pkg (500mL reconstituted)
44894	**AminoLink Plus Immobilization Kit**
66385	**Slide-A-Lyzer Dialysis Cassette Kit**, 10 dialysis cassettes, each appropriate for 0.1-0.5mL samples
66528	**Slide-A-Lyzer Concentrating Solution**, 200mL
89893	**Zeba Desalt Spin Columns, 10mL**, 5 columns, each appropriate for 0.7-4mL

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Current product instructions are available at *www.thermoscientific.com/pierce*. For a faxed copy, call 800-874-3723 or contact your local distributor.

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