INSTRUCTIONS

Thermo SCIENTIFIC

NAb™ Spin Kits, 1mL for Antibody Purification

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89978 89979 89980 89981

Number	Description
89978	**NAb Protein A Plus Spin Kit, 1mL** Binding Capacity**:** ≥ 34mg human IgG per column
89979	**NAb Protein G Spin Kit, 1mL** Binding Capacity 11-15mg human IgG per column
89980	**NAb Protein A/G Spin Kit, 1mL** Binding Capacity: ≥ 7mg human IgG per column
89981	**NAb Protein L Spin Kit, 1mL** Binding Capacity: 5-10mg human IgG per column

Kit Contents:

NAb Spin Columns, 2 each, columns are supplied with top caps and snap-off reusable bottom closures. Each column contains a 1mL resin bed of crosslinked 6% beaded agarose in 0.02% sodium azide.

Binding Buffer, 1 pack (100mM phosphate, 150mM sodium chloride; pH 7.2 when dissolved in 500mL of ultrapure water)

IgG Elution Buffer, 240mL, pH 2.8

Neutralization Buffer, 12mL, 1M Tris•HCl, pH 8.5

Storage: Upon receipt store kit at 4°C. These products are shipped at ambient temperature.

Introduction

The Thermo Scientific™ NAb™ Spin Kits are convenient for rapid, small-scale affinity purification of antibodies from a variety of sample types. Each pre-filled microcentrifuge spin column 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. Also included in each kit are buffers and a streamlined protocol for purifying at least two antibody samples.

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 of these immobilized protein resins is most appropriate. The following paragraphs provide general guidelines for making this choice; consult our 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.

Additional Materials Required

- 15mL collection tubes
- Table-top centrifuge set to 1,000 × *g* for all centrifugation steps
- Storage solution: 0.02% sodium azide in phosphate-buffered saline (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 (SAVE closure for later use). Place column in a 15mL collection tube and centrifuge for 1 minute to remove storage solution. Discard the flowthrough.
4. Equilibrate column by adding 2mL of Binding Buffer. Centrifuge for 1 minute and discard the flow-through. Repeat.
5. Reseal the column by inverting the original snap-off closure, and with a slight twisting motion, press it firmly to the bottom tip of the column. Apply sample to column and tightly cap the 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 100µL of Neutralization Buffer to three 15mL collection tubes and place the spin column into one of the tubes.
9. Add 1 ml Elution Buffer to the column 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 280nm. If required for downstream applications, exchange the buffer using Thermo Scientific™ Zeba™ Spin Desalting Columns or Slide-A-Lyzer™ Dialysis Cassettes (see Related Thermo Scientific Products Section).
11. To regenerate the column for storage or re-use, add 3 ml of Elution Buffer and centrifuge for 1 minute. Repeat. Wash column with 3 ml of PBS to remove elution buffer. Add 3 ml of storage solution, then reseal the column by inverting the original snap-off closure, and with a slight twisting motion, press it firmly to the bottom tip of the column. Cap the top 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 the 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 280nm. The last fractions should have an absorbance similar to the Binding Buffer.
7. Add 100µL 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 280nm and pool fractions as desired. If required for downstream applications, exchange the buffer using Zeba Spin Desalting Columns or 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 remains in the column, reseal the column by inverting the original snap-off bottom closure, and with a slight twisting motion, press firmly to the bottom tip of the column and secure the top cap. Store column at 4°C.

Troubleshooting

Problem	Possible Cause	Solution
No protein detected in any elution fractions by absorbance at 280nm or general protein staining of electrophoresed sample	Sample was 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 was at low concentration or had 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 Thermo Scientific AminoLink Plus Kit (see Related Products).
Antibody of interest purified, but it is denatured (as determined by lack of function in downstream assay)	Antibody was sensitive to low-pH Elution Buffer.	Try Gentle Ag/Ab Elution Buffer (see Related Thermo Scientific Products)
	Downstream application was sensitive to neutralized Elution Buffer.	Desalt or dialyze eluted sample into an application-compatible buffer.

Related Thermo Scientific Products

89896 **Pierce Centrifuge Columns, 2mL,** 25 units

89897 **Pierce Centrifuge Columns, 5mL,** 25 units

21001 **Protein A IgG Binding Buffer,** 1L

21019 **Protein G IgG Binding Buffer,** 1L

54200 **Protein A/G IgG Binding Buffer,** 240mL

21027 **Gentle Ag/Ab Elution Buffer**, 500mL

37501 **Monoclonal Antibody Isotyping Kit I (HRP/ABTS)**

44894 **AminoLink™ Plus Immobilization Kit**

66385 **Slide-A-Lyzer Dialysis Cassette Kit**

66528 **Slide-A-Lyzer Concentrating Solution**, 200mL

89893 **Zeba Spin Desalting Columns, 10mL**, 5 columns

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For descriptions of symbols on product labels or product documents, go to thermofisher.com/symbols-definition. The information in this guide is subject to change without notice.

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Revision	Date	Description
B	31 July 2024	Correcting spin column usage.
A	17 October 2015	New document for NAb™ Spin Kits, 1mL.