INSTRUCTIONS

Thermo SCIENTIFIC

NAb™ Spin Kits, 0.2mL for Antibody Purification

89948 89949 89950 89951 1939.4

Number	Description
89948	**NAb Protein A Plus Spin Kit, 0.2mL** Binding Capacity: ≥ 7mg human IgG per column
89949	**NAb Protein G Spin Kit, 0.2mL** Binding Capacity: 2.2-3.0mg human IgG per column
89950	**NAb Protein A/G Spin Kit, 0.2mL** Binding Capacity: ≥ 1.4mg human IgG per column
89951	**NAb Protein L Spin Kit, 0.2mL** Binding Capacity: 1.0-2.0mg human IgG per column

Kit Contents:

NAb Spin Columns, 10 columns, supplied with top and bottom caps; each column contains a 0.2mL resin bed of crosslinked 6% beaded agarose in 0.02% sodium azide

Microcentrifuge Tubes, 2mL, 80 tubes

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

IgG Elution Buffer, 50mL, pH 2.8

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

Storage: Upon receipt store kit at 4°C. These kits 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 100-1000µg of IgG from 25-500µL 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 sufficient collection tubes, buffers and a streamlined protocol for purifying at least 10 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 one 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 immunoglobulin classes (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

- Microcentrifuge set to 5000 × *g* for all centrifugation steps
- Storage Solution: 0.02% sodium azide in phosphate-buffered saline (PBS)

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 5000 × *g*.
2. Snap off bottom closure and loosen cap on spin column. Place column in a 2mL collection tube, centrifuge for 1 minute and discard the flow-through.
3. Equilibrate column by adding 400µL 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 25-500µL of antibody-containing sample and cap column.
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 400µL 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 40µL of Neutralization Buffer to three collection tubes and place the spin column into one of the tubes.
9. Add 400µL 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 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 used column for storage or re-use, add 400µL 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 280nm 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 such as Thermo Scientific™ AminoLink™ Plus Kit (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 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

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

- Tech Tip #34: Binding characteristics of antibody-binding proteins
- Tech Tip #43: Protein stability and storage

Related Thermo Scientific Products

89868	**Pierce™ Spin Columns,** 50 units
69720	**Pierce Microcentrifuge Tubes, 2mL,** 72 tubes
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**
89882	**Zeba Spin Desalting Columns, 0.5mL,** 25/pkg, sample volumes of 30-130 µl
89889	**Zeba Spin Desalting Columns, 2mL**, 5/pkg sample volumes 200-700 µl
66385	**Slide-A-Lyzer™ Dialysis Cassette Kit**
69576	**Slide-A-Lyzer MINI Dialysis Units Plus Float**
66528	**Slide-A-Lyzer Concentrating Solution**, 200mL

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