INSTRUCTIONS

# NAb™ Spin Columns, 5mL

## For Antibody Purification

89960 89961 89962 89963 1942.2

| Number | Description |
|---|---|
| **89960** | **NAb Protein A Plus Spin Column, 5mL**, 1 each<br>Binding Capacity: ≥ 175mg human IgG or 80-85mg mouse IgG per column |
| **89961** | **NAb Protein G Spin Column, 5mL**, 1 each<br>Binding Capacity: 55-75mg human IgG per column |
| **89962** | **NAb Protein A/G Spin Column, 5mL**, 1 each<br>Binding Capacity: ≥ 35mg human IgG per column |
| **89963** | **NAb Protein L Spin Column, 5mL**, 1 each<br>Binding Capacity: 20-50mg human IgG per column |

Contents: Columns are supplied with top and bottom caps. Each column contains a 5mL 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 rapid, small-scale affinity purification of antibodies from a variety of sample types. Each column containing 5mL of the immobilized protein resin enables quick purification of 5-65mg of IgG from 2-10mL 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 very 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

- 50mL collection tubes
- Centrifuge set to 1,000 × *g* for all centrifugation steps
- Binding Buffer: 0.1M phosphate, 0.15M sodium chloride; pH 7.2 (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 phosphate-buffered saline (PBS)

## Procedure for Antibody Purification

**Note:** Typically, the resin may be used as many as 10 times without significant loss in binding capacity.

1. Equilibrate column and buffers to room temperature. Set table top centrifuge to 1,000 × *g*.
2. Prepare sample for purification by diluting in Binding Buffer to a minimum of 5mL, or a maximum volume of 10mL.
3. Loosen top cap on spin column and snap off bottom closure. Place column in a 50mL collection tube, centrifuge for 1 minute and discard the flow-through.
4. Equilibrate column by adding 10mL of Binding Buffer. Centrifuge for 1 minute and discard the flow-through. Repeat this step once.
5. Cap bottom of column with the included rubber cap. Apply sample to column and tightly cap 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 50mL collection tube and centrifuge for 1 minute.

   **Note:** This first collection tube contains the nonbound sample components and may be analyzed to assess binding efficiency and capacity.
7. Place column in a new 50mL collection tube. Wash column by adding 10mL Binding Buffer. Centrifuge for 1 minute and collect wash fraction. Repeat wash two additional times for a total of three washes.
8. Add 500 µl of Neutralization Buffer to three 50mL collection tubes and place the spin column into one of the tubes.
9. Add 5mL of 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 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 Thermo Scientific Zeba Spin Desalting 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 10mL of Elution Buffer and centrifuge for 1 minute. Repeat once. Wash column with 10mL of Storage Solution. Add 10mL 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.

## 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 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 the 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



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