INSTRUCTIONS

Pierce Protein A/G Agarose

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20421 20422 20423 20424

Number	Description
20423	**Pierce Protein A/G Plus Agarose**, 2mL settled resin Binding capacity: 27mg mouse IgG or ≥ 50mg human IgG/mL of settled resin
20424	**Pierce Protein A/G Plus Agarose**, 20mL settled resin Binding capacity: 27mg mouse IgG or ≥ 50mg human IgG/mL of settled resin
20421	**Pierce Protein A/G Agarose,** 3mL settled resin
20422	**Pierce Protein A/G Agarose,** 15mL settled resin Binding capacity: 9mg mouse IgG or ≥ 7mg human IgG/mL of settled resin Support: Crosslinked 6% beaded agarose supplied as a 50% slurry (e.g., 3mL of settled resin is equivalent to 6mL of 50% slurry) containing 0.02% sodium azide **Storage:** Upon receipt store product at 4°C. Product is shipped at ambient temperature.

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Introduction

Thermo Scientific™ Pierce™ Protein A/G Agarose is an excellent purification tool for most immunoglobulins. Protein A/G binds to all human IgG subclasses and also binds somewhat to IgA, IgE, IgM and, to a lesser extent, IgD; therefore, it has a broader species binding range than either Protein A or Protein G individually. Unlike non-recombinant Protein G, Protein A/G does not bind serum albumin because the gene sequence coding for the albumin-binding site has been eliminated. Protein A/G is an excellent tool for purification and detection of mouse monoclonal antibodies from IgG subclasses because Protein A/G binds all mouse IgG subclasses but does not bind IgA, IgM or murine serum albumin.

Protein A/G is a genetically engineered protein (MW ~ 50,500; apparent MW by SDS-PAGE ~ 40,000-45,000) that combines the IgG binding profiles of both Protein A and Protein G. The secreted Protein A/G contains four Fc-binding domains from Protein A and two from Protein G making it a more general tool for investigating and purifying immunoglobulins. In addition, Protein A/G binding to immunoglobulins is not as pH dependent as Protein A.

Pierce Biotechnology 3747 N. Meridian Road | PO Box 117 Rockford, IL 61105 USA | (815) 968-0747 (815) 968-7316 fax | thermofisher.com

Important Product Information

- Thermo Scientific™ Protein A/G IgG Binding Buffer (Product No. 54200) and IgG Elution Buffer (Product No. 21009) have been optimized to provide the highest efficiency of IgG binding and elution for most species. Optimal immunoglobulin binding to Protein A/G is dependent on the buffer composition, not pH. Using other buffer formulations may significantly alter the binding capacity and the wash volumes required for efficient purification and, therefore, optimization may be necessary.
- The total IgG content of serum is approximately 10-15mg/mL. The concentration of antibody in tissue culture supernatant varies considerably among hybridoma clones. Be aware that antibodies from fetal bovine serum (FBS) culture media supplement will be purified along with the antibody of interest.
- For optimal recovery, use a sample size such that the expected IgG load on the column is less than 80% of the maximum binding capacity.

Column Procedure for Antibody Purification using Protein A/G

Note: The following protocol is for using a gravity-flow column packed with 1mL of Pierce Protein A/G Agarose (i.e., 2mL of the 50% slurry). When using columns containing other resin volumes, reagent amounts must be adjusted accordingly. See the Additional Information Section for batch and spin cup methods.

A. Additional Materials Required

- Column capable of containing at least 1mL resin bed volume such as the Disposable Polypropylene Columns (Product No. 29922) or the Column Trial Pack (Product No. 29925) that contains two each of three column sizes.
- Binding Buffer: (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: 1 ml of high-ionic strength alkaline buffer such as 1 M phosphate or 1 M Tris (pH 7.5-9)
- (Optional): Thermo Scientific™ Slide-A-Lyzer™ Dialysis Cassette or Zeba™ Spin Desalting Columns (Product No. 89893) for buffer exchange

B. Antibody Purification Procedure

1. Equilibrate Immobilized Protein A/G and all buffers to room temperature.
2. Carefully pack the column with 2mL of resin slurry, following the instructions provided with the columns.
3. Equilibrate the column by adding 5mL of the Binding Buffer and allowing the solution to drain through the column.

   Note: To avoid air bubbles being drawn into the resin, remove the top cap before the bottom cap when opening column.
4. Dilute sample at least 1:1 with Binding Buffer before application to the Protein A/G Column to maintain the proper ionic strength and pH for optimal binding.

   Note: Plasma may become hazy upon dilution with the Binding Buffer because of lipoprotein precipitation. Centrifuge the diluted sample at 10,000 × *g* for 20 minutes and apply the supernatant to the equilibrated Immobilized Protein A/G.
5. Apply the diluted sample to the column and allow it to flow completely into the resin. Do not allow the resin bed to run dry. Any volume may be applied provided the total amount of antibody is less than 80% of column capacity.

   Note: If the sample contains more IgG than can bind to the Protein A/G column (or is an antibody type that does not bind to Protein A/G), the flow-through will contain excess antibody. By saving the flow-through, non-bound antibody can be recovered and examined by antibody-specific assays.
6. Wash the Protein A/G column with 15mL of the Binding Buffer.

   Note: If desired, verify that all non-bound proteins are removed from the column by collecting separate 2mL fractions as the solution drains and measuring their absorbance at 280nm. The last fractions should have absorbances similar to Binding Buffer alone.
7. Elute antibodies with 5mL of Elution Buffer and collect 0.5-1mL fractions. Immediately adjust eluted fractions to physiologic pH by adding 100µL of the Neutralization Buffer per 1mL of eluate. Monitor the elution by measuring the absorbance at 280nm or by protein assay such as the Thermo Scientific™ Pierce™ BCA Protein Assay Kit (Product No. 23225).

8. Pool the eluted IgG fractions that contain the highest absorbance. The purified antibodies may be used directly for SDS-PAGE, or the buffer may be exchanged by dialysis or desalting column to one that is compatible with the specific downstream application (see Related Thermo Scientific Products).
9. Regenerate column by washing with 12mL of Elution Buffer. Columns may be regenerated at least 10 times without significant loss of binding capacity.
10. For storage, wash column with 5mL of water containing 0.02% sodium azide. When approximately 3mL of solution remains, replace the bottom cap followed by the top cap on the column. Store columns upright at 4°C.

Example Immunoprecipitation (IP) Procedure using Protein A/G

A. Additional Materials Required

- 1.5-2mL microcentrifuge tube
- IP Buffer: 25mM Tris, 150mM NaCl; pH 7.2 (Thermo Scientific™ BupH™ Tris Buffered Saline Pack, Product No. 28379)
- Antigen Sample: Antigen-containing lysate or sample prepared in IP Buffer or other buffer that is compatible with both the desired antibody binding interaction and the binding of antibody to Protein A/G
- Elution Buffer: IgG Elution Buffer (Product No. 21004) or 0.1-0.2M glycine•HCl buffer, pH 2.5-3.0
- Electrophoresis Loading Buffer: Lane Marker Reducing Sample Buffer (5X), (Product No. 39000)
- Neutralization Buffer (optional): 1mL of strong alkaline buffer, such as 1M phosphate or 1M Tris, (pH 7.5-9)

B. Immunoprecipitation Procedure

Note: This procedure uses 50µL of settled Immobilized Protein A/G (100µL resin slurry). This amount of resin is sufficient to bind 25-250µg of antibody. Depending on the amount of antibody needed to immunoprecipitate the desired amount of antigen, scale the amount of resin and suggested wash and elution volumes accordingly. To allow for proper mixing, make sure the total reaction volume does not completely fill the microcentrifuge tube.

1. In a microcentrifuge tube, combine 50-1000µL of the Antigen Sample and the optimized amount of antibody. Incubate the reaction overnight at 4°C.
2. Add 100µL of Immobilized Protein A/G resin slurry to a new microcentrifuge tube and briefly centrifuge to pellet resin. Discard the supernatant.
3. Add 0.5mL of IP Buffer, briefly centrifuge and discard supernatant. Repeat this step two times.
4. Add the antigen-antibody complex to the resin and incubate reaction with gentle mixing for 2 hours at room temperature.
5. Add 0.5mL of IP Buffer, briefly centrifuge and discard supernatant. Repeat this step several times.
6. To elute the immune complex, add 50µL of Elution Buffer and incubate for 5 minutes. Briefly centrifuge the tube and collect the supernatant. Repeat this step and combine the two supernatant fractions.

   Alternatively, wash the complex-bound resin with 0.5mL water, briefly centrifuge and discard supernatant. Add Electrophoresis Loading Buffer to the complex-bound resin and incubate for 5 minutes at 95°C. Briefly centrifuge the tube, collect the supernatant and evaluate by SDS-PAGE.
7. Adjust eluate to physiological pH by adding ~10µL of the Neutralization Buffer per 100µL of eluate. The IP products may be used directly for SDS-PAGE, or the buffer may be exchanged by dialysis or desalting column to one that is compatible with the specific downstream application (see Related Thermo Scientific Products).

Troubleshooting

Problem	Possible Cause	Solution
Flow of the column is exceedingly slow (i.e., < 0.5mL/minute)	Outgassing of buffers or sample on the column, resulting in blockage of resin pores with microscopic air bubbles	Degas buffers and remove air bubbles from column (see Additional Information section for suggested Tech Tip protocol)
Considerable antibody purified, but no specific antibody of interest detected	Antibody of interest at very low concentration	Use serum-free medium for cell supernatant samples
		Affinity purify the antibody using the specific antigen coupled to an a affinity support such as Thermo Scientific AminoLink Plus Immobilization Kit (Product No. 44894)
Antibody of interest purified, but it is degraded (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 suitable buffer

Additional Information Available on the Web

- Tech Tip #34: Binding characteristics for Immunoglobulin Binding Proteins (Protein A, G, A/G and L)
- Tech Tip #4: Batch and spin cup methods for affinity purification of proteins
- Tech Tip #13: Pack resin into polypropylene columns
- Tech Tip #7: Remove air bubbles from columns
- Tech Tip 29: Degas solutions for use in affinity columns
- Tech Tip #43: Protein stability and storage

Related Thermo Scientific Products

26146 **Pierce Classic IP Kit,** sufficient to perform 50 reactions

26147 **Pierce Crosslink IP Kit,** sufficient to perform 50 reactions

26148 **Pierce Direct IP Kit,** sufficient to perform 50 reactions

26149 **Pierce Co-IP Kit,** sufficient to perform 50 reactions

88802 **Pierce Protein A/G Magnetic Beads,** 1mL

88803 **Pierce Protein A/G Magnetic Beads,** 5mL

88804 **Pierce Protein A/G Magnetic IP/Co-IP Kit,** sufficient to perform 40 reactions

General Reference

Eliasson, M., *et al.* (1989). Differential IgG-binding characteristics of staphylococcal protein A, streptococcal protein G, and a chimeric Protein AG. *J Immun* **142:**575-81.

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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