INSTRUCTIONS

Pierce® Protein A Agarose

0037.6

Number	Description
20333	**Pierce Protein A Agarose,** 5mL settled resin
20334	**Pierce Protein A Agarose,** 25mL settled resin
	Support: Crosslinked 6% beaded agarose supplied as a 50% slurry (e.g., 5mL of settled resin is equivalent to 10mL of 50% slurry) containing 0.02% sodium azide
	Binding capacity: 12-19mg of human IgG or 6-8mg mouse IgG per mL of settled resin
22810	**Pierce Protein A Plus Agarose,** 1mL settled resin
22811	**Pierce Protein A Plus Agarose,** 5mL settled resin
22812	**Pierce Protein A Plus Agarose,** 25mL settled resin
	Support: Crosslinked 6% beaded agarose supplied as a 50% slurry (e.g., 5mL of settled resin is equivalent to 10mL of 50% slurry) containing 0.02% sodium azide
	Binding capacity: > 34mg human IgG or 16-17mg mouse IgG per mL of settled resin
20338	**Protein A Trisacryl® Resin,** 5mL settled resin
	Support: Trisacryl GF-2000 supplied as a 50% slurry (e.g., 5mL of settled resin is equivalent to 10mL of 50% slurry) containing 0.02% sodium azide
	Binding capacity: 15mg human IgG or 6-8mg mouse IgG per mL of settled resin
	Storage: Upon receipt store product at 4-8°C. Product is shipped at ambient temperature.

Introduction

Protein A is a cell wall component produced by several strains of *Staphylococcus aureus* that consists of a single polypeptide chain (MW ~ 46,700; MW by SDS-PAGE ~ 42,000) and contains little or no carbohydrate.[1,2] Protein A has four high affinity ($K_a = 10^8$/M) binding sites capable of binding specifically to the Fc region of immunoglobulin molecules from several species.[3] The Protein A molecule is heat-stable and retains its native conformation when exposed to denaturing reagents such as 4M urea, 4M thiocyanate and 6M guanidine hydrochloride.[4]

Covalently immobilized Protein A matrices have been extensively used to purify IgG from several species of mammals. However, the interaction between Protein A and IgG is not equivalent for all species.[5,6] Even within a species, Protein A interacts with some subgroups of IgG and not others.[7,8] There are also many instances in which monoclonal antibodies do not bind, such as the majority of rat immunoglobulins. Despite its variable binding characteristics, Protein A possesses properties that make it ideal for isolation of IgG. When using samples from species not well referenced in the literature, preliminary experiments should be conducted to determine if a particular immunoglobulin binds.

We developed a system to increase the IgG yields from a variety of species. The Thermo Scientific Pierce Protein A Agarose is prepared using a coupling method that results in excellent resin stability and binding characteristics. Using our optimized binding buffers enhances IgG binding, resulting in higher yields compared with conventional methods.[9,10]

Important Product Information

- Our buffers provide the highest efficiency of IgG binding and elution for most species. Using other buffer formulations may significantly alter the binding capacity and the wash volumes required for efficient purification. Therefore, optimization may be necessary when using other buffers.
- 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. The total IgG content of serum is approximately 10-15mg/mL (see page 1 for binding capacities). 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.
- The Trisacryl GF-2000 Support is a rigid matrix composed of an acrylamide monomer. The matrix can withstand up to 2-3 bars of pressure, has excellent resistance to microbial contamination and can tolerate pH extremes (i.e., 1 to 11). Trisacryl GF-2000 Support is also compatible with commonly used denaturants, organic solvents and detergents.
- The crosslinked 6% beaded agarose support can tolerate commonly used water-miscible solvents when they are added in a stepwise gradual manner. The agarose support will compress under pressure causing column flows to slow. Never freeze agarose supports, as this will cause irreversible damage to the bead structure.
- Serum samples, ascites fluid, plasma or tissue culture supernatant may be used with this product.

Column Procedure for Antibody Purification Using Protein A Agarose

Note: The following protocol is for using a gravity-flow column packed with 1mL of Protein A 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: Protein A IgG Binding Buffer (Product No. 21001 or 21007)
- Elution Buffer: IgG Elution Buffer (Product No. 21004 or 21009) or 0.1M glycine, pH 2-3
- Neutralization Buffer: 1mL of high-ionic strength alkaline buffer such as 1M phosphate or 1M 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 the Protein A agarose 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 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 Protein A agarose.
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 column (or is an antibody type that does not bind to Protein A), 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 column with 15mL of the Binding Buffer.

   Note: If desired, verify that all non-bound proteins are removed by collecting separate 2mL fractions as the solution drains and measuring their absorbance at 280nm. The last fractions should have absorbencies similar to Binding Buffer.

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 Thermo Scientific 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 Agarose

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
- 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 Protein A agarose (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 Protein A resin slurry to the antigen-antibody complex. Incubate reaction with gentle mixing for 2 hours at room temperature.
3. Add 0.5mL of IP Buffer, centrifuge for 2-3 minutes at 2500 × *g* and discard supernatant. Repeat this step several times.
4. To elute the immune complex, add 50µL of Elution Buffer and incubate for 5 minutes. Centrifuge tube for 1-3 minutes at 2500 × *g* and collect the supernatant. Repeat this step and combine the two supernatant fractions.

   Alternatively, wash the complex-bound resin with 0.5mL water, centrifuge for 2-3 minutes at 2500 × *g*, and discard supernatant. Add Electrophoresis Loading Buffer to the complex-bound resin and incubate for 5 minutes at 95°C. Centrifuge the resin mixture at 2500 × *g*, collect the supernatant and evaluate by SDS-PAGE.
5. 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).

Additional Information Available from our Web Site

- 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 #7: Remove air bubbles from columns
- Tech Tip #29: Degas solutions for use in affinity columns
- Tech Tip #43: Protein stability and storage

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 has blocked 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 is at 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 was purified but 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
No protein detected in any elution fraction	Sample devoid of antibody species or isotype that binds to Protein A	Refer to the Binding Characteristics Table for Protein A (see Additional Information section)
Antibody bands interfere with antigen detection after immunoprecipitation	Antibody is eluted along with the antigen	Crosslink antibody to the Protein A by using the Thermo Scientific Pierce Crosslink IP Kit (Product No. 26147)

Related Thermo Scientific Products

44667 **Protein A IgG Purification Kit**
89924 **Pierce Protein A Chromatography Cartridges, 1mL,** 2/pkg
89925 **Pierce Protein A Chromatography Cartridges, 5mL,** 1/pkg
89948 **NAb Protein A Plus Spin Kit, 0.2mL,** 10 column kit
53139 **Protein A UltraLink Resin,** 5mL
53142 **Protein A Plus UltraLink Resin,** 5mL

General References

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2. Sjoquist, J., *et al.* (1972). Protein A isolated from *Staphylococcus aureus* after digestion with lysostaphin. *Eur J Biochem* **29:**572-8.
3. Hjelm, H., *et al.* (1975). Immunologically active and structurally similar fragments of protein A from *Staphylococcus aureus*. *Eur J Biochem* **57:**395-403.
4. Sjoholm, I. (1975). Protein A from *Staphylococcus aureus*: spectropolarimetric and spectrophotometric studies. *Eur J Biochem* **51:**55-61.
5. Kronvall, G. and Williams, R.C. (1969). Differences in anti-protein A activity among IgG subgroups. *J Immunol* **103(4):**828-33.
6. Surolia, A., *et al.* (1982). Protein A: Nature's universal anti-body *TIBS* **7:**74-6.
7. Kronvall, G., *et al.* (1970). Protein A reactivity with mouse immunoglobulins. *J Immunol* **105:**1116-23.
8. Ey, P.L., *et al.* (1978). Isolation of pure IgG_1, IgG_{2a}, and IgG_{2b} immunoglobulins from mouse serum using protein A-Sepharose. *Immunochem* **15:**429-36.
9. Bigbee, W.L., *et al.* (1983). Monoclonal antibodies specific for the M- and N-Forms of human glycophorin A. *Mol Immunol* **20:**1353-62.

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