

Immobilized Protein A

0037.4

Number	Description
20333	Immobilized Protein A , 5 ml settled resin
20334	Immobilized Protein A , 25 ml settled resin Support: Crosslinked 6% beaded agarose supplied as a 50% slurry (e.g., 5 ml of settled resin is equivalent to 10 ml of 50% slurry) containing 0.02% sodium azide Binding capacity: 12-19 mg of human IgG or 6-8 mg mouse IgG per ml of settled resin
22810	Immobilized Protein A Plus , 1 ml settled resin
22811	Immobilized Protein A Plus , 5 ml settled resin
22812	Immobilized Protein A Plus , 25 ml settled resin Support: Crosslinked 6% beaded agarose supplied as a 50% slurry (e.g., 5 ml of settled resin is equivalent to 10 ml of 50% slurry) containing 0.02% sodium azide Binding capacity: > 34 mg human IgG or 16-17 mg mouse IgG per ml of settled resin
20338	Trisacryl[®] Immobilized Protein A , 5 ml settled resin Support: Trisacryl GF-2000 supplied as a 50% slurry (e.g., 5 ml of settled resin is equivalent to 10 ml of 50% slurry) containing 0.02% sodium azide Binding capacity: 15 mg human IgG or 6-8 mg 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 4 M urea, 4 M thiocyanate and 6 M guanidine hydrochloride.⁴

Covalently immobilized Protein A matrices have been extensively used to purify IgG from several species of mammals.⁵⁻⁷ However, the interaction between Protein A and IgG is not equivalent for all species.^{8,9} Even within a species, Protein A interacts with some subgroups of IgG and not others.¹⁰ 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.

Pierce developed a system to increase the IgG yields from a variety of species. The Immobilized Protein A is prepared using a coupling method that results in excellent resin stability and binding characteristics. Using the optimized Pierce binding buffers enhance IgG binding, resulting in higher yields compared with conventional methods.^{12,13}

Important Product Information

- Pierce 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 10-15 mg/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., 1-11). 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 Immobilized Protein A

Note: The following protocol is for using a gravity-flow column packed with 1 ml of Immobilized Protein A (i.e., 2 ml 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 1 ml 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 0.1 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: Slide-A-Lyzer[®] Dialysis Cassette or Zeba[™] Desalt Spin Columns (Product No. 89893) for buffer exchange

B. Antibody Purification Procedure

1. Equilibrate Immobilized Protein A and all buffers to room temperature.
2. Carefully pack the column with 2 ml of resin slurry, following the instructions provided with the columns.
3. Equilibrate the column by adding 5 ml 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 $10,000 \times g$ for 20 minutes and apply the supernatant to the equilibrated Immobilized Protein A.

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 15 ml of the Binding Buffer.

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

7. Elute antibodies with 5 ml of Elution Buffer and collect 0.5-1 ml fractions. Immediately adjust eluted fractions to physiologic pH by adding 100 μ l of the Neutralization Buffer per 1 ml of eluate. Monitor the elution by measuring the absorbance at 280 nm or by protein assay such as 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 Pierce Products).
9. Regenerate column by washing with 12 ml of Elution Buffer. Columns may be regenerated at least 10 times without significant loss of binding capacity.
10. For storage, wash column with 5 ml of water containing 0.02% sodium azide. When approximately 3 ml 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 Immobilized Protein A

A. Additional Materials Required

- 1.5-2 ml microcentrifuge tube
- IP Buffer: 25 mM Tris, 150 mM NaCl; pH 7.2 (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 0.1-0.2 M glycine•HCl buffer, pH 2.5-3.0
- Electrophoresis Loading Buffer: Lane Marker Reducing Sample Buffer (5X), (Product No. 39000)
- Neutralization Buffer (optional): 1 ml of strong alkaline buffer, such as 1 M phosphate or 1 M Tris, (pH 7.5-9)

B. Immunoprecipitation Procedure

Note: This procedure uses 50 μ l of settled Immobilized Protein A (100 μ l resin slurry). This amount of resin is sufficient to bind 25-250 μ g 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 50-1,000 μ l of the Antigen Sample and the optimized amount of antibody. Incubate the reaction overnight at 4°C.
2. Add 100 μ l of Immobilized Protein A resin slurry to the antigen-antibody complex.
3. Incubate reaction with gentle mixing for 2 hours at room temperature.
4. Add 0.5 ml of IP Buffer, centrifuge for 2-3 minutes at 2,500 \times g and discard supernatant. Repeat this step several times.
5. To elute the immune complex, add 50 μ l of Elution Buffer and incubate for 5 minutes. Centrifuge tube for 1-3 minutes at 2,500 \times g and collect the supernatant. Repeat this step and combine the two supernatant fractions.

Alternatively, wash the complex-bound resin with 0.5 ml water, centrifuge for 2-3 minutes at 2,500 \times 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 2,500 \times g, collect the supernatant and evaluate by SDS-PAGE.

6. Adjust eluate to physiological pH by adding ~10 μ l of the Neutralization Buffer per 100 μ l 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 Pierce Products).

Additional Information Available from the Pierce Website

- 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 gel (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

Troubleshooting

Problem	Possible Cause	Solution
Flow of the column is exceedingly slow (i.e., < 0.5 ml/minute)	Outgassing of buffers or sample on the column resulted 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 is at low concentration	Use serum-free medium for cell supernatant samples
		Affinity purify the antibody using the specific antigen coupled to an affinity support such as 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 Pierce 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 Seize® X Protein A Immunoprecipitation Kit (Product No. 45215)

Related Pierce Products

66382	Slide-A-Lyzer Dialysis Cassette Kit , 10 dialysis cassettes, each appropriate for 0.5-3.0 ml samples
69576	Slide-A-Lyzer MINI Dialysis Units , 10 units plus float, each appropriate for 10-100 µl samples
89893	Zeba Desalt Spin Columns, 10 ml , 5 columns, each appropriate for 700-4,000 µl samples
89882	Zeba Desalt Spin Columns, 0.5 ml , 25 columns, each appropriate for 30-130 µl samples
21027	Gentle Ag/Ab Elution Buffer , 500 ml
45215	Seize X Protein A Immunoprecipitation Kit

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