INSTRUCTIONS

Pierce® Protein A Columns

20356

0566.4

Number	Description
20356	**Pierce Protein A Columns,** 5 × 1mL settled resin, pre-packed columns Support: Crosslinked, 6% beaded agarose in 0.02% sodium azide solution Binding Capacity: 12-19mg human 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

The Thermo Scientific Pierce Protein A Columns consist of high-quality Protein A agarose resin pre-packed in columns that provide for easy affinity purification of polyclonal antibodies from rabbit and other species. The Immobilized Protein A is prepared using a coupling method that results in excellent gel stability and binding characteristics. The columns are intended for traditional gravity-flow procedures (See Related Thermo Scientific Products Section for other formats of columns and kits).

Protein A is a cell wall component produced by several strains of *Staphylococcus aureus* that consists of a single polypeptide chain of molecular weight 42,000 and contains little or no carbohydrate.[1,2] Protein A bind specifically to the Fc region of immunoglobulin molecules, especially IgG. The Protein A molecule contains four high affinity ($K_a = 10^8$/M) binding sites capable of interacting with the Fc region of several species.[3] The 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]

Immobilized Protein A has been used extensively for the isolation of 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. For instance, human IgG_1, IgG_2 and IgG_4 bind strongly, whereas IgG_3 does not bind[3] and mouse IgG_1 binds poorly to Protein A.[10] There are also many instances in which monoclonal antibodies do not bind to Protein A such as the majority of rat immunoglobulins.

Despite its variable binding characteristics, Protein A possesses IgG binding properties that make it ideal for affinity purification of IgG. The potential limitations of Protein A-IgG interactions must be considered when using samples from species not well referenced in the literature. When in doubt, conduct preliminary experiments to determine if a particular immunoglobulin will bind. Certain buffers enhance IgG binding compared to conventional methods (see protocol).[12,13]

Important Product Information

- The following procedure has been developed for the optimal isolation and purification of human or mouse IgG. Thermo Scientific Pierce Binding and Elution Buffers have been optimized to 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 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. 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 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 high pressure causing column flow to slow. Centrifuging the support at medium speed (i.e., 1000-2000 × *g*) for 1-3 minutes will not harm the support. Never freeze the agarose support, as this will cause irreversible damage to the bead structure.
- Clarified serum samples, ascites fluid, plasma or tissue culture supernatant may be used with this product.

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Gravity-flow Column Procedure for Antibody Purification

A. Additional Materials Required

- Binding Buffer: For best results, use Protein A IgG Binding Buffer (Product No. 21001 or 21007). Alternatively, use phosphate-buffered saline (PBS: 0.1M sodium phosphate, 0.15M sodium chloride, pH 7.2-8.0).
- 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 8.5-9
- Dialysis cassette or desalting columns (See Related Thermo Scientific Products Section) for optional buffer exchange following elution

B. Antibody Purification Procedure

1. Equilibrate Protein A column and all buffers to room temperature.
2. 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: When using plasma, the sample will become hazy after dilution with the Binding Buffer because of the lipoprotein precipitation. Centrifuge the diluted sample at 10,000 × *g* for 20 minutes and apply the supernatant to the equilibrated immobilized Protein A.
3. To avoid air bubbles being drawn into the resin bed, open a Protein A column by removing the top cap first. Empty the storage solution (contains 0.02% sodium azide).
4. Remove the bottom cap from the column. Equilibrate the column by adding 5mL of the Binding Buffer and allowing the solution to drain through the column.
5. Apply the diluted sample to the column and allow it to flow completely into the resin bed. The column will stop flowing automatically when the liquid level reaches the top porous disc. 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 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 a 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 to a system compatible with the specific downstream application (see optional procedure that follows).
9. Regenerate column by washing with 12mL of Elution Buffer.
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. Columns may be regenerated a minimum of 10 times without significant loss of binding capacity.

Procedure for Buffer Exchange of the Eluted Antibody (Optional)

In the purification procedure, IgG is eluted from Protein A using a low-pH, amine-containing buffer and then neutralized by addition of sodium phosphate or Tris. Often, this neutralized buffer is suitable for long-term storage of the antibody and will not interfere in downstream applications. However, certain applications will require a buffer exchange. For example, most biotinylation or enzyme-labeling procedures for the antibody will necessitate removal of amines from the buffer.

Two options exist for buffer exchange. One option is to dialyze the purified antibody against the buffer of choice. For this purpose, choose a Thermo Scientific Slide-A-Lyzer Dialysis Cassette (See Related Thermo Scientific Products Section) appropriate for the volume of purified antibody solution. Use Slide-A-Lyzer® Concentrating Solution to concentrate the antibody solution after dialysis.

The second option for buffer exchange is size-exclusion chromatography or gel filtration such as Thermo Scientific Zeba Spin Desalting Columns (See Related Thermo Scientific Products Section). The porous resin matrix has an average molecular weight exclusion limit of 7000. Therefore, molecules greater than 7kDa will pass through the column in the void volume, while smaller molecules will pass through more slowly as they migrate through the entire resin bed.

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, which results in blockage of gel 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 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 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 using Thermo Scientific Pierce Gentle Ag/Ab Elution Buffer (See Related Thermo Scientific Products Section)
	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)

Additional Information

Please visit the website for additional information relating to this product including the following items:

- Tech Tip # 4: Batch and spin cup methods for affinity purification of proteins
- Tech Tip # 7: Remove air bubbles from columns to restore flow rate
- Tech Tip # 29: Degas buffers for use in affinity and gel filtration columns
- Tech Tip # 34: Binding characteristics of Protein A, Protein G, Protein A/G and Protein L
- Tech Tip # 43: Protein stability and storage

Related Thermo Scientific Products

89952, 89948	**NAb™ Protein A Plus Spin Columns, Kit**, 10 × 0.2mL
89956, 89978	**NAb Protein A Plus Spin Columns, Kit**, 5 × 1mL
89960	**NAb Protein A Plus Spin Columns**, 1 × 5mL
89924	**Protein A Chromatography Cartridges**, 2 × 1mL
89925	**Protein A Chromatography Cartridges**, 1 × 5mL
66382	**Slide-A-Lyzer Dialysis Cassette Kit**, 10 dialysis cassettes, each appropriate for 0.5-3.0mL samples
66526	**Slide-A-Lyzer Concentrating Solution**, 10 × 15mL
89893	**Zeba Spin Desalting Columns**, 5 × 10mL, each appropriate for 0.7-4.0mL samples
21027	**Gentle Ag/Ab Elution Buffer**, 500mL
44894	**AminoLink® Plus Immobilization Kit**
37503	**Pierce Rapid ELISA Mouse mAb Isotyping Kit**

References

1. Bjork, L., *et al.* (1972). Some physicochemical properties of protein A from *Staphylococcus aureus*. *Eur J Biochem* **29:**579-84.
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. Goding, J.W. (1978). Use of Staphylococcal protein A as an immunological reagent. *J Immunol Methods* **20:**241-53
6. Lindmark, R., *et al.* (1983). Binding of Immunoglobulins to protein A and immunoglobulin levels in mammalian sera. *J Immunol Methods* **62:**1-13.
7. Kronvall, G. (1970). Phylogenetic insight into evolution of mammalian Fc fragment of gamma G globulin using staphylococcal protein A. *J Immunol* **104:**140-7.
8. Kronvall, G. and Williams, R.C. (1969). Differences in anti-protein A activity among IgG subgroups. *J Immunol* **103(4):**828-33.
9. Surolia, A., *et al.* (1982). Protein A: Nature's universal anti-body. *TIBS* **7:**74-6.
10. Kronvall, G., *et al.* (1970). Protein A reactivity with mouse immunoglobulins. *J Immunol* **105:**1116-23.
11. Skvaril, F. (1976). The question of specificity in binding human IgG subclasses to protein A-Sepharose. *Immunochem* **13:**871-2.
12. 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.
13. 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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