INSTRUCTIONS

Pierce™ Protein G Agarose

20397 20398 20399 22851 22852

0576.6

Number	Description
20398	**Pierce Protein G Agarose,** 2mL settled resin
20399	**Pierce Protein G Agarose,** 10mL settled resin
20397	**Pierce Protein G Agarose,** 25mL settled resin
	Support: Crosslinked 6% beaded agarose supplied as 50% slurry (e.g., 2mL of settled resin is equivalent to 4mL of 50% slurry) in 0.02% sodium azide
	Binding Capacity: 11-15mg human IgG per mL of settled resin
22851	**Pierce Protein G Plus Agarose,** 2mL settled resin
22852	**Pierce Protein G Plus Agarose,** 10mL settled resin
	Support: Crosslinked 6% beaded agarose supplied as 50% slurry (e.g., 2mL of settled resin is equivalent to 4mL of 50% slurry) in 0.02% sodium azide
	Binding Capacity: > 20mg human IgG per mL of settled resin

Storage: Upon receipt store product at 4°C. Product is shipped at ambient temperature.

Table of Contents

Introduction

The Thermo Scientific™ Pierce™ Protein G Agarose is useful for purification antibodies and immunoprecipitation (IP). Samples containing IgG are incubated with Protein G agarose in a buffer that facilitates binding. After non-IgG and non-antigen components of the sample are washed from the resin, and the bound IgG and antigen may be recovered by elution.

Protein G, a bacterial cell wall protein isolated from group G *Streptococci*,[1-3] binds to mammalian IgGs mainly through Fc regions. Native Protein G has two IgG binding domains and also sites for albumin and cell-surface binding.[4-9] Albumin and cell-surface binding domains have been eliminated from recombinant Protein G to reduce nonspecific binding. Consequently, recombinant Protein G can be used to separate IgG from crude human or mouse IgG serum samples.

Although the tertiary structures of Protein A and Protein G are very similar, their amino acid compositions differ significantly, resulting in different binding characteristics.[6-9] Protein G may be used for purification of mammalian monoclonal and polyclonal IgGs that do not bind well to Protein A. Protein G has greater affinity than Protein A for most mammalian IgGs, especially for certain subclasses including human IgG_3, mouse IgG_1 and rat IgG_{2a}.[1,2,3,6] Unlike Protein A, Protein G does not bind to human IgM, IgD and IgA.[3,6,9]

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

Important Product Information

- Optimal immunoglobulin binding to Protein G is dependent on the buffer composition. Pierce 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 efficient purification and, therefore, optimization may be necessary. See Product Description Section on page 1 for binding capacities.
- For optimal recovery, use a sample size such that the expected IgG load is less than 80% of the maximum binding capacity. The total IgG content of serum is ~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.

Column Procedure for Antibody Purification Using Protein G Agarose

Note: The following protocol is for using a gravity-flow column packed with 1mL of Protein 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: Protein G IgG Binding Buffer (Product No. 21011)
- 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 G 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 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 Protein G 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 G column (or is an antibody type that does not bind to Protein 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 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 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 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 G Agarose

A. Additional Materials Required

- 1.5-2mL microcentrifuge tube
- IP Buffer: 25mM Tris, 150mM NaCl; pH 7.2 (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 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 Protein G 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 G agarose 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).

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 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 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)

Continued next page

Continued from previous page

Problem	Possible Cause	Solution
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
No antibody detected in any elution fraction	Sample devoid of antibody species or subclass that binds to Protein G	Refer to the Binding Characteristics Table for Protein G (see Additional Information Section)

Additional Information Available on our Web site

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

Related Thermo Scientific Products

66382 **Slide-A-Lyzer Dialysis Cassette Kit**

69576 **Slide-A-Lyzer MINI Dialysis Units**

89893 **Zeba Spin Desalting Columns, 7K MWCO, 10mL**

89882 **Zeba Spin Desalting Columns, 7K MWCO, 0.5mL**

21027 **Gentle Ag/Ab Elution Buffer**, 500mL

Cited References

1. Akerstrom, B. and Bjorck, L. (1986). A physicochemical study of protein G, a molecule with unique immunoglobulin G-binding properties. *J Biol Chem* **261:**10240-7.
2. Akerstrom, B., *et al.* (1985). Protein G: A powerful tool for binding and detection of monoclonal and polyclonal antibodies. *J Immunol* **135:**2589-92.
3. Bjorck, L. and Kronvall, G. (1984). Purification and some properties of streptococcal protein G, a novel IgG-binding reagent. *J Immunol* **133:**969-74.
4. Akerstrom, B., *et al.* (1987). Definition of IgG and albumin binding regions of streptococcal protein G. *J Biol Chem* **262:**13388-91.
5. Sjobring, U., *et al.* (1988). Isolation and characterization of a 14-kDa albumin-binding fragment of streptococcal protein G. *J Immunol* **140:**1595-9.
6. Fahnestock, S. (1987). Cloned streptococcal protein G genes. *TIBS* **5**:79-83.
7. Olsson, A., *et al.* (1987). Structure and evolution of the repetitive gene encoding streptococcal protein G. *Eur J Biochem* **168:**319-24.
8. Fahnestock, S., *et al.* (1986). Gene for an immunoglobulin-binding protein from a group G *streptococcus. J Bacteriol* **167:**870-80.
9. Guss, B., *et al.* (1986). Structure of the IgG-binding regions of streptococcal protein G. *EMBO J* **5:**1567-75.