INSTRUCTIONS

Pierce™ Protein L Agarose

20510 20512

0778.6

Number	Description
20510	**Pierce Protein L Agarose,** 2mL settled resin
20512	**Pierce Protein L 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) containing 0.02% sodium azide

Binding Capacity: ~5-10mg human IgG/mL of resin

Storage: Upon receipt store product at 4-8°C. Product is shipped at ambient temperature.

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Introduction

Protein L is an immunoglobulin-binding protein that was isolated from the bacteria *Peptostreptococcus magnus* and is now produced recombinantly. Protein L binds to immunoglobulin kappa light chains without interfering with the antigen-binding site and binds a wider range of Ig classes and subclasses than other antibody-binding proteins such as Protein A or Protein G. Protein L binds to all classes of Ig (i.e., IgG, IgM, IgA, IgE and IgD). Protein L also binds single chain variable fragments (Scfv) and Fab fragments. The Thermo Scientific Pierce Protein L Agarose is prepared using a leak-resistant coupling method that ensures excellent resin stability and binding characteristics.

Important Product Information

- Protein L **only** binds to immunoglobulins containing light chains of type kappa I, III, and IV in human and kappa I in mouse. Protein L also may be specific for certain kappa subgroups in other species. Protein L binds scfv without interfering with antigen binding.
- Protein L binds weakly to rabbit immunoglobulins and does not bind immunoglobulins from bovine, goat or sheep; nor does it bind to lambda light chains.
- The total IgG content of serum is approximately 10-15 mg/ml and, therefore, 2 ml of settled resin has only enough binding capacity to purify antibody from ~1 ml of serum. The concentration of antibody in tissue culture supernatant varies considerably among hybridoma clones.
- The described purification procedure is for human IgG. For optimal recovery, use a sample size such that the expected Ig load on the column is less than 80% of the maximum binding capacity.
- Serum samples, ascites fluid, plasma or tissue culture supernatant may be used with this product.

Gravity-flow Column Procedure for Antibody Purification

Note: The following protocol is for using a column packed with 2mL of Pierce Protein L Agarose. When using columns containing other resin volumes, reagent amounts must be adjusted accordingly.

A. Additional Materials Required

- Disposable column capable of containing at least 2mL resin bed volume such as the Disposable Polypropylene Columns (Product No. 29922) or the Column Trial Pack (Product No. 29925), which contains two each of three column sizes.
- Binding Buffer: 0.1M phosphate, 0.15M sodium chloride; pH 7.2 (Product No. 28372)
- Elution Buffer: 0.1M glycine, pH 2-3 or Pierce IgG Elution Buffer (Product No. 21004 and 21009)
- Neutralization Buffer: 1mL of high-ionic strength alkaline buffer such as 1M phosphate or 1M Tris, (pH 7.5-9)
- Thermo Scientific Slide-A-Lyzer Dialysis Cassette or Zeba Spin Desalting Column (for example, Product No. 89894) for buffer exchange

B. Immunoglobulin Purification Procedure

1. Equilibrate the Protein L agarose and reagents to room temperature before use.
2. Carefully pack the Protein L resin slurry (4mL) into the column (see Additional Information Section for the Tech Tip Protocol). Equilibrate the Protein L column by adding 10mL of the Binding Buffer and allowing the solution to drain through the column.
3. Dilute sample at least 1:1 with Binding Buffer before applying onto the Protein L column to maintain optimal ionic strength and pH for binding.

   Note: If plasma is used, the sample may appear hazy after adding the Binding Buffer caused by lipoprotein precipitation. For optimal Ig recoveries, centrifuge the diluted sample at 10,000 × *g* for 20 minutes and apply the supernatant to the equilibrated Protein L column.
4. Apply up to 4mL of the diluted sample to the column and allow it to flow completely into the resin. The column will stop flowing automatically when the liquid level reaches the top disc. Larger volumes may be applied provided the total amount of Ig is less than 80% of column capacity.

   Note: If the sample contains more Ig than can bind to the Protein L column (or is an antibody type that does not bind to Protein L), the flow-through will contain excess antibody. By saving the flow-through, non-bound antibody can be recovered and examined by antibody-specific assays.
5. Wash the Protein L column with 10-15mL of 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.
6. Elute antibodies with 6-10mL of Elution Buffer and collect 0.5-1mL fractions. Immediately adjust eluted fractions to physiologic pH by adding 100µL of the Neutralization Buffer to 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).
7. Pool the eluted Ig 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).
8. Regenerate column by washing with 12mL of Elution Buffer.
9. 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.

   Note: If required, perform a buffer exchange using a Slide-A-Lyzer Dialysis Cassette or a Zeba™ Spin Desalting Column.

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 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)
No protein detected in any elution fractions	Sample was devoid of antibody species or isotype that binds to Protein L	Use Protein A, Protein G or Protein A/G resin
Considerable antibody purified, but no specific antibody of interest detected	Antibody of interest was 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 (Product No. 44894)
Antibody of interest purified, but it is degraded (as determined by lack of function in downstream assay)	Antibody was sensitive to low-pH Elution Buffer	Try Thermo Scientific Gentle Ag/Ab Elution Buffer (see Related Products)
	Downstream application was sensitive to neutralized Elution Buffer	Desalt or dialyze eluted sample into suitable buffer

Additional Information

Visit our website for additional information including the following items:

- Tech Tip #13: Pack beaded affinity resin into columns
- 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

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

66528 **Slide-A-Lyzer Concentrating Solution**, 200mL

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

44894 **AminoLink™ Plus Immobilization Kit**

37503 **Pierce Rapid ELISA Mouse mAb Isotyping Kit**

20421 **Pierce Protein A/G Agarose,** 3mL settled resin with column sample kit

20422 **Pierce Protein A/G Agarose,** 15mL settled resin with column sample kit

20398 **Pierce Protein G Agarose,** 2mL settled resin

20399 **Pierce Protein G Agarose,** 10mL settled resin

20333 **Pierce Protein A Agarose,** 5mL settled resin

20334 **Pierce Protein A Agarose,** 25mL settled resin

General References

Åkerström, B. and Björck, L. (1989). Protein L - an immunoglobulin light chain-binding bacterial protein. Characterization of binding and physicochemical properties. *J Biol Chem* **264:**19740-6.

Beckingham, J.A., *et al.* (1999). Interactions between a single immunoglobulin-binding domain of protein L from *Peptostreptococcus magnus* and a human κ light chain. *Biochem J* **340:**193-9.

Björck, L. (1988). Protein L - a novel bacterial cell wall protein with affinity for immunoglobulin light chains. *J Immunol* **140:**1194-7.

Enokizono, J., *et al*. (1997). NMR Analysis of the interaction between protein L and Ig light chains. *J Mol Biol* **270:**8-13.

Kastern, W., *et al*. (1992). Structure of *peptostreptococcal* protein L and identification of a repeated immunoglobulin light chain-binding domain. *J Biol Chem* **267:**12820-5.

Nilson, B.H.K., *et al.* (1993). Purification of antibodies using protein L-binding framework structures in the light chain variable domain. *J Immul Meth* **164:**33-40.

Product References

Kinoshita, Y., *et al.* (2001). Pescadillo, a novel cell cycle regulatory protein abnormally expressed in malignant cells. *J Biol Chem* **276(9):**6656-65.

Nozawa, K., *et al.* (2001). Preferential blockade of CD8+ T cell responses by administration of Anti-CD137 ligand monoclonal antibody results in differential effect on development of murine acute and chronic graft-versus-host diseases. *J Immunol* **167:**4981-6.

Current product instructions are available at *www.thermoscientific.com/pierce*. For a faxed copy, call 800-874-3723 or contact your local distributor.