

Pierce[™] Protein L Plus Agarose

20520

0777.5

Number	Description
20520	Pierce Protein L Plus Agarose , 2mL of settled resin Support: Crosslinked 6% beaded agarose supplied as a 50% slurry (e.g., 2mL of settled resin is equivalent to 4mL of 50% slurry) containing 0.02% sodium azide Binding Capacity: ~10-20mg human IgG/mL of resin

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

Table of Contents

Introduction	1
Important Product Information	1
Gravity-flow Column Procedure for Antibody Purification Using Protein L Agarose.....	2
A. Additional Materials Required	2
B. Immunoglobulin Purification Procedure	2
Troubleshooting.....	3
Additional Information	3
Related Thermo Scientific Products	3
General References	4
Product References	4

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. Thermo Scientific Pierce Protein L Plus 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 10-15mg/mL and, therefore, 2mL of settled resin has only enough binding capacity to purify antibody from ~1-2mL 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 Using Protein L Agarose

Note: The following protocol is for using a column packed with 2mL of 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 NaCl; pH 7.2 (Product No. 28372)
- Elution Buffer: 0.1M glycine, pH 2-3 or 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 (e.g., Product No. 66382) or Zeba Spin Desalting Column (for example, Product No. 89894) for optional buffer exchange

B. Immunoglobulin Purification Procedure

1. Equilibrate 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 $10,000 \times g$ for 20 minutes and apply the supernatant to the equilibrated Protein L column.

4. Apply up to 4mL of diluted serum 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 100 μ L of the Neutralization Buffer per 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 using a Slide-A-Lyzer™ Dialysis Cassette or a Zeba™ Spin Desalting Column.
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.

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 fraction	Sample was devoid of antibody species or isotype that binds to Protein L	Use Protein A, Protein G or Protein A/G agarose
Considerable antibody purified, but no specific antibody of interest detected	Antibody of interest was at very 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 Thermo Scientific 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 was sensitive to low-pH Elution Buffer	Try Gentle Ag/Ab Elution Buffer (see Related Thermo Scientific Products)
	Downstream application was sensitive to neutralized Elution Buffer	Desalt or dialyze eluted sample into suitable buffer

Additional Information

Visit our web site for additional information on this product 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, 10 × 15mL
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 A/G Agarose, 2mL settled resin
20399	Pierce Protein A/G Agarose, 10mL settled resin
20333	Pierce Protein A/G Agarose, 5mL settled resin
20334	Pierce Protein A/G Agarose, 25mL settled resin

General References

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