INSTRUCTIONS

Pierce™ Chromatography Cartridges Protein L

89928 89929

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Number	Description
89928	**Pierce Chromatography Cartridges Protein L,** 2 × 1mL
89929	**Pierce Chromatography Cartridges Protein L,** 1 × 5mL

Binding Capacity: 4-5mg human IgG/mL of resin bed

Note: Protein L is immobilized on crosslinked 6% beaded agarose supplied in 0.05% sodium azide in water. Each product is supplied with an accessory pack (1 female Luer-Lok™ Adapter, 1 connector fitting, 1 column plug and 1 or 2 bottom caps).

Storage: Upon receipt store at 4-8°C. Product is shipped at ambient temperature. Do not freeze.

Introduction

The Thermo Scientific™ Pierce™ Chromatography Cartridges Protein L are convenient, ready-to-use prepacked devices for isolation and purification of immunoglobulin classes IgG, IgM, IgA, IgE and IgD via their kappa light chains. Protein L binds to certain subtypes of antibody kappa light chains, predominant in humans and mice, without interfering with antigen binding sites. Typically, Protein L cartridges are used for purifying monoclonal antibodies from ascites or cell culture supernatants. Protein L does not bind bovine antibodies, eliminating contamination from bovine immunoglobins when purifying serum-supplemented cell culture supernatants. Protein L also binds single chain variable fragments (scFv) and Fab fragments, unlike Protein A, Protein G or Protein A/G.

Pierce Chromatography Cartridges are compatible with the major automated liquid-chromatography systems or for manual syringe processing (see Table 1 for general properties of the cartridges). The cartridges attach directly to ÄKTA™ or FPLC Systems without additional connectors. An accessory pack, included with each product, readily adapts columns for use with Luer-Lok Syringe Fittings or 1/16" tubing. Protein L cartridges provide fast, easy and reproducible chromatographic separations and can be regenerated for multiple uses.

Table 1. Properties of the Pierce Chromatography Cartridges Protein L.

Support	Crosslinked 6% beaded agarose
Ligand	Immobilized Recombinant Protein L, 45,800 MW
Binding Capacity	4-5mg human IgG/mL of resin bed
Cartridge Dimensions	0.7 × 2.7cm (1mL column); 1.3 × 3.8cm (5mL column)
Particle Size	45-165µm
Void Volume	0.32ml (1mL column); 1.5mL (5mL column)
Recommended Flow Rate	1mL/min (1ml column); 1-2mL/min (5mL column)
Maximum Recommended Flow Rate	4mL/min (1mL column); 5mL/min (5mL column)
pH Limits	3-10
Maximum Operating Pressure	0.3MPa, 43.5psi or 3bar
Cartridge Material	Polypropylene
Frit	Polyethylene, 10µm
Storage Solution	0.02-0.05% Sodium azide
Accessory Pack	Luer-Lok Adapter to 10-32 male Finger-tight 10-32 connector fitting for 1/16" OD tubing Column plug for 10-32 coned port Cap 1/16 male

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

- Use high purity buffers prepared with high quality water. For best results, degas or filter buffers through a 0.45µm filter.
- Serum samples, ascites fluid, plasma or tissue culture supernatant may be used with this product. The interaction between antibodies and Protein L is not equivalent for all species or for all subgroups of IgG. Refer to Tech Tip: Binding Characteristics for Immunoglobulin Proteins and Proteins L, A, G, and A/G for further information on species and subgroup specificities of Protein L.
- For optimal recovery, use a sample size such that the expected IgG load on the resin 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.
- Do not freeze the resin as this will cause irreversible damage to the bead structure.
- Pierce Cartridges may be used singly or connected in series (2-3 columns) to increase capacity. Back pressure will be greater when columns are used in a series than when used as single columns.
- To monitor protein as it emerges from the column, measure the UV absorbance at 280nm.

Additional Materials Required

- Suitable liquid chromatography system (LC procedure only) with 1/16" tubing or syringes (syringe procedure only)
- Binding Buffer: 100mM sodium phosphate, 150mM sodium chloride, pH 7.2. Alternatively, prepare a Thermo Scientific™ BupH™ Phosphate Buffered Saline Pack (Product No. 28372) with high-purity water according to instructions. Sodium azide (0.02%) may be added to the buffer solution as a preservative. For best results, process the buffer through a 0.45µm filter before use in LC applications.
- Elution Buffer: 0.1M glycine, pH 2-3, or Pierce IgG Elution Buffer (Product No. 21004). Sodium azide (0.02%) may be added to the buffer solution as a preservative. For best results, process the buffer through a 0.45µm filter before use in LC applications.
- Neutralization Buffer: High ionic strength alkaline buffer such as 1M phosphate or 1M Tris at pH 7.5-9
- Pierce Chromatography Cartridge Desalting (Product No. 89934 or 89935), Thermo Scientific™ Zeba™ Desalting Column (Product No. 89891, 89892, 89893 or 89894) or Slide-A-Lyzer™ Dialysis Cassettes for optional buffer exchange
- Additional connectors and fittings are required to attach to the Bio-Rad BioLogic™ System.

Procedure for Antibody Purification using a Liquid Chromatography System

1. Equilibrate the cartridge and all buffers to room temperature. Ensure all solutions are degassed.
2. Prepare the LC system by filling tubing with buffer. Remove top plug from column and carefully snap off the end-tab (do not twist). To avoid introducing air into the system, let a few drops of buffer flow from tubing into column top then connect cartridge top to the tubing; allow a few drops to emerge from the column before connecting to the LC inlet port.
3. Equilibrate the column with 5-10 column volumes of the Binding Buffer at a flow rate of 1mL/minute for the 1mL column or 5mL/minute for the 5mL column.
4. For maximum binding, adjust the sample to the ionic strength and pH of the Binding Buffer by diluting it at least 1:1 before applying to the column. Alternatively, buffer-exchange the sample against the Binding Buffer. If the sample contains insoluble matter, centrifuge or filter (0.45µm filter) it before use.

   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 column.
5. Apply the diluted sample to the column. For maximum binding, apply at a flow rate of 1mL/minute for the 1mL column and 1-2mL/minute for the 5mL column. Apply any volume of sample provided the total amount of antibody is < 80% of resin capacity.

   Note: If the sample exceeds the binding capacity of the resin (or contains an antibody type that does not bind), non-bound antibody can be recovered in the flow-through and analyzed by antibody-specific assays.

6. Wash the resin with 5-10 column volumes of Binding Buffer or until the absorbance approaches baseline.
7. Elute with approximately 2-5 column volumes of Elution Buffer and collect 0.5-1mL fractions. Immediately adjust eluted fractions to physiological pH by adding 100µL of the Neutralization Buffer per 1mL of eluate. Alternatively, add the Neutralization Buffer to the collection tubes before eluting.
8. Analyze the purified fractions directly by SDS-PAGE, or dialyze or desalt the sample into a buffer that is compatible with the specific downstream application.
9. Resin may be regenerated and reused multiple times without significant loss of binding capacity. To prevent cross-contamination, reuse columns with identical antibodies. Regenerate the resin with 3-5 column volumes of Elution Buffer and re-equilibrate with Binding Buffer until the pH returns to the buffer value.
10. For storage, wash the resin with five column volumes of water containing 0.02-0.05% sodium azide. Attach supplied bottom cap followed by the top plug. Store the cartridge at 4°C.

Procedure for Antibody Purification using a Syringe

Note: The void volumes are 0.320mL for the 1mL columns and 1.5mL for the 5mL columns.

1. Equilibrate the cartridge and all solutions to room temperature. Ensure all solutions are degassed.
2. Fill a syringe with 5-10 column volumes of buffer.
3. Attach the syringe to the Luer-Lok Adapter included in the accessory pack. Remove top plug from the cartridge and carefully snap off the end-tab. To avoid introducing air into the system, allow a few drops to emerge from the Luer-Lok Adapter and then connect to the cartridge top. Securely tighten the connection.
4. Equilibrate the resin with 5-10 column volumes of buffer at a flow rate of ~1mL/minute for the 1mL column or ~5mL/minute for 5mL column. Remove syringe from the Luer-Lok Adapter.
5. For maximum binding, adjust the sample to the ionic strength and pH of the Binding Buffer by diluting it at least 1:1 before applying to the column. Alternatively, buffer-exchange the sample against the Binding Buffer. If the sample contains insoluble matter, centrifuge or filter (0.45µm filter) it before use.

   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 column.
6. Fill an appropriately sized syringe with the diluted sample and connect it to the Luer-Lok Adapter. Depress the syringe plunger to pass the sample through the column. For maximum binding, apply at a flow rate of 1mL/minute for the 1mL column and 1-2mL/minute for the 5mL column. Any volume may be applied provided the total amount of antibody is less than 80% of the resin capacity.

   Note: If the sample exceeds the binding capacity of the resin (or contains an antibody type that does not bind), non-bound antibody can be recovered in the flow-through and analyzed by antibody-specific assays.
7. Change the syringe and wash the resin with 5-10 column volumes of Binding Buffer.
8. Change syringe and elute with approximately 2-5 column volumes of Elution Buffer and collect 0.5-1mL fractions. Immediately adjust eluted fractions to physiological pH by adding 100µL of the Neutralization Buffer per 1mL of eluate. Alternatively, add the Neutralization Buffer to the collection tube before eluting.
9. Analyze the purified fractions directly by SDS-PAGE, or dialyze or desalt the sample into a buffer that is compatible with the specific downstream application.
10. Columns may be regenerated and reused multiple times without significant loss of binding capacity. To prevent cross-contamination, reuse columns with identical antibodies. Regenerate the resin in 3-5 column volumes of Elution Buffer and re-equilibrate in Binding Buffer until the pH returns to the buffer value.
11. For storage, wash the resin with five column volumes of water containing 0.02-0.05% sodium azide. Attach supplied bottom cap followed by the top plug. Store the column at 4°C.

Troubleshooting

Problem	Possible Cause	Solution
Sample in flow-through fractions	Exceeded resin's binding capacity	Make sure the total amount of protein loaded is < 80% of the resin's binding capacity
	Sample or resin not at proper pH or correct ionic strength for optimum binding	Dilute sample 1:1 with Binding Buffer or perform a buffer exchange so the ionic strength and pH is optimal for binding and make sure to equilibrate the resin with the Binding Buffer
	Sample being applied too fast for proper binding	Slow the flow rate to 0.1-1mL/minute during sample loading
No antibody detected in any elution fraction	Sample devoid of antibody species or subclass that binds to Protein L	Refer to our catalog or website to select the best antibody-binding protein for a specific species
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
		Use the specific antigen coupled to an a affinity support such as Thermo Scientific™ AminoLink™ Plus Immobilization Kit (Product No. 44894) to purify the antibody
Antibody of interest purified, but it is degraded (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

Additional Information Available on Our Website

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

Related Thermo Scientific Products

89971 **Accessory Pack** (1 female Luer-Lok Adapter, 1 connector fitting, 1 column plug and 1 bottom cap)

28372 **BupH™ Phosphate Buffered Saline Pack,** 40 packs

21004 **IgG Elution Buffer,** 1L

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

89935 **Pierce Chromatography Cartridge Desalting,** 5 × 5mL

89806 **Protein Stabilizing Cocktail** (4X), 10mL

General References

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

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

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