INSTRUCTIONS

Pierce® Thiophilic Adsorbent

20500

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Number	Description
20500	**Pierce Thiophilic Adsorbent,** 10mL resin supplied as a 50% slurry (i.e., 20mL total volume) in 500mM Tris buffer pH 7.4 with sodium azide as a preservative Support: Crosslinked, 6% beaded agarose Bead Diameter: 45-165µm Binding Capacity: ~20mg of human IgG/mL of settled resin **Storage:** Upon receipt store at 4°C. Do not freeze. Product is shipped at ambient temperature.

Introduction

The Thermo Scientific Pierce Thiophilic Adsorbent allows for simple, rapid, one-step immunoglobulin purification from a wide variety of serum, ascites or tissue culture supernatant samples. Immunoglobulin purification using Thiophilic Adsorbent ("T-Gel") is based on the ability of some proteins to bind to a ligand that contains a sulfone group in proximity to a thioether group (Figure 1). The binding event is a highly selective type of lyotropic salt-promoted interaction.

Thiophilic adsorption has some elements of both hydrophobic and hydrophilic adsorption. Increased non-chaotropic salts promote both thiophilic and hydrophobic interactions. However, hydrophobic interaction chromatography is strongly promoted by high concentrations of sodium chloride, whereas thiophilic adsorption is not. Salts that interact with water molecules, such as potassium sulfate and ammonium sulfate, promote protein binding to thiophilic supports.

Pierce Thiophilic Adsorbent has a high binding capacity and a broad specificity toward immunoglobulins from various species regardless of the immunoglobulin type or subclass. This method provides a low cost, efficient alternative to ammonium sulfate precipitation as the first step of a multi-step immunoglobulin purification scheme for crude samples. The adsorbent exhibits high protein recovery with excellent preservation of antibody activity. The gentle elution conditions yield concentrated, essentially salt-free, highly purified immunoglobulins at near-neutral pH. Thus, this simple one-step method eliminates the need for additional treatment of the sample for storage or for subsequent conjugation reactions.

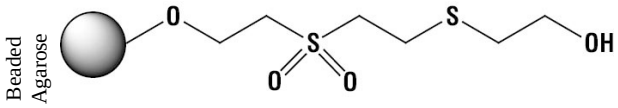

Figure 1. Molecular structure of the immobilized ligand that comprises Thiophilic Adsorbent.

Important Product Information

- Temperature, pH, ionic strength and specific salts affect binding and elution efficiency and sample purity. High concentrations of non-chaotropic salts improve coupling efficiency, but chaotropic salts that do not form structures with water decrease coupling efficiency.
- Coupling at pH < 8 will generally increase protein binding; however, greater amounts of proteins other than immunoglobulins will also bind to the support.
- When 1mL of sera is applied to a 3mL column of Thiophilic Adsorbent, essentially all immunoglobulins present will bind. However, at larger sample volumes, one or more of the non-bound (NB) fractions will contain immunoglobulins. These NB fractions may be pooled and treated as a sample for a subsequent purification to recover all immunoglobulins from the original sample.

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Additional Materials Required

- Crystalline Potassium sulfate, ACS Reagent Grade
- Binding Buffer: 0.5M potassium sulfate, 50mM sodium phosphate; pH 8.0 with 0.05% sodium azide as a preservative
- Elution Buffer: 50mM sodium phosphate pH 8.0 with 0.05% sodium azide as a preservative

  Note: If subsequent application involves conjugation with peroxidase, omit the sodium azide.
- Storage Buffer: 0.5M Tris•HCl pH 7.4 with 0.02% sodium azide
- Regeneration Solution: 8M guanidine•HCl (Product No. 24115)
- Empty column to pack desired bed volume of Thiophilic Adsorbent (e.g., Pierce Centrifuge Columns, Product No. 89896, 89897 and 89898)

Material Preparation

Sample Preparation	While mixing, add 87mg of potassium sulfate per milliliter of sample for a final concentration of 0.5M potassium sulfate. Gently mix sample to avoid denaturation of the immunoglobulins. When the potassium salt is fully dissolved, centrifuge sample at 10,000 × *g* for 20 minutes. Carefully remove the clear supernatant and filter it using a 0.5µm filter.
Regeneration Solution	Add 124mL ultrapure water to 230g of crystals to prepare 300mL of the 8M reagent. Stir at room temperature until completely dissolved. Solubilization of guanidine•HCl is endothermic and may require mild warming in a 37°C water bath to completely dissolve. Allow the reagent to equilibrate to room temperature before using. Store solution at 4°C for up to one year.

Procedure for Immunoglobulin Purification using Thiophilic Adsorbent

Note: The following procedure assumes use of a 5mL column (Product No. 89897) that has been packed with 3mL of Thiophilic Adsorbent. Adjust procedure accordingly for other column sizes or styles.

1. Equilibrate Thiophilic Adsorbent Column, buffers and sample(s) to room temperature.
2. Remove top and bottom caps from column. (When uncapping column throughout procedure, always remove the top cap before the bottom cap to prevent air bubbles from being drawn into resin bed.)
3. Place column in 16 × 150mm test tube or other holder and allow storage solution to drain from column. (Throughout the procedure, always add more buffer to the column as soon as solution drains down to top of the resin bed or cap the column bottom.)
4. Equilibrate column with 12mL of Binding Buffer. Discard flow-through.
5. Apply the sample to the column and allow the sample to completely enter the resin bed. Column flow will cease when the liquid level reaches the top disc. If desired, collect the column effluent as 3mL non-bound (NB) fractions.
6. Wash the column with up to 5-10 resin-bed volumes of Binding Buffer. Monitor absorbance of the fractions at 280nm to determine when all NB material is washed from the column.
7. Elute immunoglobulins with 12 resin-bed volumes of Elution Buffer and collect the effluent as 3mL fractions. Measure the absorbance of each fraction at 280nm vs. water.
8. Regenerate the Thiophilic Adsorbent by adding five resin-bed volumes of Regeneration Solution to the column and allowing the column to drain.

   Note: The Regeneration Solution completely removes all residual proteins from the Thiophilic Adsorbent; however, to avoid the possibility of cross-contaminating samples, dedicate each column for a particular application.
9. Rinse column with 10 bed volumes of degassed ultrapure water followed by three (3) bed volumes of storage buffer. When about 3mL of storage buffer remains above the resin bed, cap the column (bottom then top) and store column upright at 4°C.

Related Thermo Scientific Products

37503	**Pierce Rapid ELISA Mouse mAb Isotyping Kit**
23310	**Easy-Titer® Human IgG Assay Kit**
23300	**Easy-Titer Mouse IgG Assay Kit**
23305	**Easy-Titer Rabbit IgG Assay Kit**
44887	**Pierce IgM Fragmentation Kit**
53027	**FITC Antibody Labeling Kit**
53031	**Rhodamine Antibody Labeling Kit**
45206	**Melon™ Gel IgG Spin Purification Kit**

Product References

Harsay, E. and Schekman, R. (2002). A subset of yeast vacuolar protein sorting mutants is blocked in one branch of the exocytic pathway. *J Cell Biol* **156(2):**271-85.

Koustova, E. *et al.* (2001). LP-BM5 virus-infected mice produce activating autoantibodies to the AMPA receptor. *J Clin Invest* **107(6):**737-44.

Suh, J.S., *et al.* (1998). Antibodies from patients with heparin-induced thrombocytopenia/thrombosis recognize different epitopes on heparin: platelet factor 4. *Blood* **91(3):**916-22.

General References

Porath, J., *et al*. (1985). Thiophilic adsorption—a new method for protein fractionation. *FEBS Lett* **185:**306-10.

Hutchens, T.W. and Porath, J. (1986). Thiophilic adsorption of immunoglobulins—analysis of conditions optimal for selective immobilization and purification. *Anal Biochem* **159:**217-26.

Belew, M., *et al.* (1987). A one-step purification method for monoclonal antibodies based on salt-promoted adsorption chromatography on a 'thiophilic' adsorbent. *J Immunol Meth* **102:**173-82.

Nopper, B., *et al*. (1989). A thiophilic adsorbent for the one-step high-performance liquid chromatography purification of monoclonal antibodies. *Anal Biochem* **180:**66-71.

Oscarsson, S., *et al.* (1991). Thiophilic adsorbents for RIA and ELISA procedures. *J Immunol Meth* **143:**143-9.

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