Pierce® Thiophilic Adsorption Kit

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**Kit Contents:**

**Thiophilic Adsorbent Columns,** 4 × 3 ml prepacked columns containing immobilized thiophilic adsorbent ligand on 6% beaded agarose resin (45-165 µm diameter beads);

**Binding Capacity:** ~20 mg of human IgG/ml of settled resin

**Binding Buffer,** 1 L, contains 0.5 M potassium sulfate, 50 mM sodium phosphate, 0.05% sodium azide; pH 8.0

**Elution Buffer,** 1 L, contains 50 mM sodium phosphate, 0.05% sodium azide; pH 8.0

**Column Storage Buffer (2X),** 100 ml, contains 1 M Tris and 0.05% sodium azide; pH 7.4

**Guanidine Hydrochloride,** 230 g, sufficient reagent to prepare 300 ml of an 8 M solution

**Column Accessories,** white tips (5) and column extenders (4)

**Storage:** Upon receipt store kit at 4°C. Do not freeze Thiophilic Adsorbent. Product is shipped at ambient temperature.

**Introduction**

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.](image-url)
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 1 ml of sera is applied to a 3 ml 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.

Additional Materials Required

- Crystalline Potassium sulfate, ACS Reagent Grade

Material Preparation

Sample Preparation  While mixing, add 87 mg of potassium sulfate per milliliter of sample for a final concentration of 0.5 M potassium sulfate. Gently mix sample to avoid denaturation of the immunoglobulins. When the potassium salt is fully dissolved, centrifuge sample at 10,000 x g for 20 minutes. Carefully remove the clear supernatant and filter it using a 0.5 μm filter.

Regeneration Solution  Add 124 ml ultrapure water to 230 g of crystals to prepare 300 ml of the 8 M 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 4°C for up to one year.

Procedure for Immunoglobulin Purification using Thiophilic Adsorbent

1. Equilibrate Thiophilic Adsorbent Column, buffers and sample(s) to room temperature.

2. Remove top cap and twist off bottom tab 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 × 150 mm 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 with a supplied white tip.)

4. Equilibrate column with 12 ml 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 3 ml 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 280 nm 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 3 ml fractions. Measure the absorbance of each fraction at 280 nm 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 3 ml of storage buffer remains above the resin bed, cap the column (use supplied white tip for bottom cap) and store column upright at 4°C.
Related Pierce Products

- 37501 Monoclonal Antibody Isotyping Kit 1 (HRP/ABTS)
- 23310 Easy-Titer® Human IgG Assay Kit
- 23300 Easy-Titer Mouse IgG Assay Kit
- 23305 Easy-Titer Rabbit IgG Assay Kit
- 44887 IgM Fragmentation Kit
- 53004 Fluorescein Isothiocyanate (FITC) Labeling Kit
- 53002 Rhodamine Labeling Kit
- 45206 Melon™ Gel IgG Spin Purification Kit

Product References


General References


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