

T-Gel™ Purification Kit

44916

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| Number | Description |
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| 44916 | T-Gel™ Purification Kit Kit Contents: T-Gel™ Adsorbent Columns , 4 x 3 ml, contains T-Gel™ Storage Buffer Support: 6% beaded agarose Bead Diameter: 45-165 µm Binding Capacity: ~20 mg of human IgG/ml of settled gel T-Gel™ Binding Buffer , 1 L, contains 0.5 M potassium sulfate, 50 mM sodium phosphate, 0.05% sodium azide; pH 8.0 T-Gel™ Elution Buffer , 1 L, contains 50 mM sodium phosphate, 0.05% sodium azide; pH 8.0 T-Gel™ 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 Extenders , 4 each |

Storage: Upon receipt store product at 4°C. Do not allow gel to freeze. Product is shipped at ambient temperature.

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Introduction

T-Gel™ Adsorbent allows for simple, rapid, one-step immunoglobulin purification from a wide variety of serum, ascites or tissue culture supernatant samples. Immunoglobulin purification using T-Gel™ Adsorbent 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). This binding event is termed thiophilic adsorption and 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.

T-Gel™ 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 T-Gel™ 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.

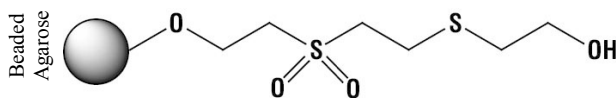


Figure 1. Molecular structure of the T-Gel™ Adsorbent immobilized ligand.

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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 T-Gel™ 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

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| 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 T-Gel™ Adsorbent

1. Equilibrate T-Gel™ Adsorbent, buffers and sample(s) to room temperature.
2. To prevent air bubbles from forming in the gel bed and below the porous discs, open a T-Gel™ Adsorbent column by first removing the top cap and then the bottom cap. Allow the storage buffer to drain.
3. Equilibrate column with 12 ml of Binding Buffer.
4. Apply the sample to the column and allow the sample to completely enter the gel. The 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.
5. Wash the column with up to 13 column volumes of Binding Buffer. Monitor absorbance of the fractions at 280 nm to determine when all NB material is washed from the column.
6. Elute immunoglobulins with 12 column volumes of Elution Buffer and collect the effluent as 3 ml fractions. Measure the absorbance of each fraction at 280 nm vs. water.
7. Regenerate the T-Gel™ Adsorbent by adding five column volumes of Regeneration Solution to the column and allowing the column to drain. Rinse column with 10 column volumes of degassed ultrapure water followed by two column volumes of storage buffer. Allow the column to drain.
Note: The Regeneration Solution completely removes all residual proteins from the T-Gel™ Adsorbent; however, to avoid the possibility of cross-contaminating samples, dedicate each column for a particular application.
8. Place the bottom cap on the column, add 3 ml of storage buffer and apply the top cap to the column. Store column upright at 4°C.

Related Pierce Products

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| 37501 | ImmunoPure[®] 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 | ImmunoPure[®] IgM Fragmentation Kit |
| 53004 | EZ-Label[™] Fluorescein Isothiocyanate (FITC) Protein Labeling Kit |
| 53002 | EZ-Label[™] Rhodamine Protein Labeling Kit |

Product References

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General References

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