INSTRUCTIONS

Pierce Albumin/IgG Removal Kit

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89875

Number	Description
89875	**Pierce Albumin/IgG Removal Kit,** sufficient to process 25 samples of ~10µL serum
	Kit Contents:
	Immobilized Cibacron Blue/Protein A, 2.2mL settled gel
	Support: Beaded agarose supplied as a 50% slurry (4.4mL total volume) in 0.02% sodium azide
	Binding/Wash Buffer, 4.25mL, contains 25mM Tris, 25mM NaCl, 0.01% sodium azide; pH 7.5
	Spin Columns and Accessories, 27 columns, caps and plugs
	Storage: Upon receipt store at 4°C. Product shipped with an ice pack. Do not freeze product.

Introduction

The Thermo Scientific™ Pierce™ Albumin/IgG Removal kit removes human serum albumin (HSA) and the major subclasses of gamma globulin (IgG) from serum, plasma or spinal fluids. This kit uses a classical mixed bed of cibacron blue/Protein A gel for an economical means for sample preparation. Analysis of serum and plasma is complicated by high concentrations of albumin and gamma globulins that can make up more than 70% of total serum protein. Removal of these proteins is often essential for the study of serum proteins. The Pierce Albumin/IgG Removal Kit allows samples to be processed in less than 40 minutes using a convenient disposable spin format. The processed sample is compatible with downstream applications such as Western blotting, 2D electrophoresis or 2D-LC mass spectrometry.

Important Product Information

- This kit is compatible with human, monkey, swine and rabbit samples.
- Because of the high concentration of albumin present in serum, each 170µL of slurry can bind sufficient albumin and IgG to process ~10µL (600µg) of serum. However, the amount of HSA and IgG in plasma or other fluid samples will vary considerably. For best results, optimize the ratio of sample to gel volume for each specific application.
- Binding of albumin and IgG will occur using a pH range from 6.0 to 9.0. However, for proper albumin binding, sample must be free of excess salts. If sample contains ≥500mM NaCl, desalt or dialyze into a low-salt buffer.
- Tween™-20 Detergent or similar detergent added to the Binding/Wash Buffer at a final concentration of ~0.01% may reduce nonspecific binding. However, detergents are not compatible with all types of applications and may reduce binding capacity for albumin.
- 1.5-2.0mL microcentrifuge tubes are required for sample collection (two per sample).

Procedure for HSA and IgG Removal

A. Resin Preparation

1. Place one spin column into a microcentrifuge tube for each sample to be processed.
2. Swirl the bottle containing the Immobilized Cibacron Blue/Protein A Gel to obtain an even suspension. The gel slurry must be homogeneous before pipetting.
3. To ensure proper gel slurry dispensing, use a wide bore or cut pipette tip. Add 170µL of gel slurry into the spin column.
4. Place column in to a microcentrifuge collection tube and centrifuge at 10,000 × *g* for 1 minute. Discard storage buffer. Insert plug into bottom of column containing the gel bed.

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B. Sample Processing

1. Dilute sample to 75µL with Binding/Wash Buffer.
2. Add the diluted sample to the gel bed and cap column. Briefly vortex column to form a suspension. Place column into a microcentrifuge tube.
3. Incubate for 10 minutes at room temperature on an orbital shaker using a mixing speed sufficient to keep the mixture suspended, or vortex every few minutes.
4. Remove bottom plug from the column and loosen cap. Return column to microcentrifuge tube and centrifuge at 10,000 × *g* for 1 minute.
5. Recover filtrate and reapply to resin bed. Cap and re-plug column.
6. Incubate for 10 minutes at room temperature on an orbital shaker or vortex every few minutes.
7. Remove bottom plug from the column and loosen cap. Return column to the tube and centrifuge at 10,000 × *g* for 1 minute to collect filtrate.
8. Add 75µL of Binding/Wash Buffer to the gel bed and centrifuge at 10,000 × *g* for 1 minute to collect wash in same tube as the filtrate from Step 7.
9. Filtrate contains sample with albumin and IgG removed. The sample can be used for immediate analysis or for further processing (i.e., desalting and concentrating), or it may be stored for later use.
10. Discard spin column containing gel or proceed to Section C. Optional Elution Protocols.

C. Optional Elution Protocols

1. Wash the gel by adding 100µL of Binding/Wash Buffer to gel bed. Centrifuge 10,000 × *g* for 1 minute in a new microcentrifuge tube.
2. Transfer column to a new collection tube and add 100µL of either of the following elution agents as appropriate for the downstream application:
   - 1D SDS-PAGE: 1X SDS Sample Buffer free of reducing agents
   - 2D Electrophoresis: 1X 2D electrophoresis buffer free of reducing agents and ampholytes
   - Native proteins: IgG Elution Buffer (Product No. 21004) or Gentle Ag/Ab Elution Buffer (Product No. 21030)
3. Incubate for 10 minutes. Centrifuge at 10,000 × *g* for 1 minute to collect eluted fraction.

Troubleshooting

Problem	Possible Cause	Solution
Incomplete removal of albumin or IgG	Sample exceeds binding capacity	Reduce amount of sample processed
	Incomplete binding	Increase incubation time
		Insure sample and gel remain mixed during incubation
	Incompatible substance in sample	Dialyze or desalt sample before processing

Related Thermo Scientific Products

89871	**In-Gel Tryptic Digestion Kit**
89849	**Polyacrylamide Spin Desalting Columns, 7K MWCO, 0.7mL,** 25/pkg
69705	**Pierce Spin Columns – ScrewCap,** 25/pkg
24612	**Pierce Silver Stain Kit**
24592	**GelCode™ Blue Stain Reagent,** 3.5L

Current product instructions are available at thermofisher.com. For a faxed copy, call 800-874-3723 or contact your local distributor.

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