INSTRUCTIONS

Pierce™ Albumin Depletion Kit

85160

2518.0

Number	Description
85160	**Pierce Albumin Depletion Kit,** contains sufficient resin and buffer for 24 spin columns

Kit Contents:

Pierce Albumin Depletion Resin, 10mL, supplied as 50% resin slurry

Binding Capacity: 2.0mg of human serum albumin (HSA) for 200µL of settled resin

Binding/Wash Buffer (25mM Tris, 75mM NaCl; pH 7.5)**,** 11mL

Pierce Spin Columns, 24 columns

Storage: Upon receipt store at 4°C. Product shipped with an ice pack.

Introduction

The Thermo Scientific Pierce Albumin Depletion Kit improves serum component analysis by rapidly removing abundant albumin protein from serum samples. The Pierce Albumin Depletion Resin provided in the kit is a high capacity, immobilized Cibacron Blue dye agarose resin, which binds a variety of species-specific albumins. Each aliquot of 200µL settled resin can process 5-50µL of serum sample in less than 10 minutes by low-speed centrifugation using the Pierce Spin Columns provided in the kit. The kit has been optimized for HSA, but also effectively binds swine, sheep and rabbit serum albumin. With modification of the binding buffer, Pierce Albumin Depletion Resin can bind bovine, calf, goat and rat albumin; however, this product is not for use with mouse albumin.

Important Product Information

- Use a clarified (centrifuged or filtered) protein solution to ensure proper resin flow.
- Albumin binding occurs at pH 6.0-9.0. Samples must be free of excess salt and, therefore, methods for preparing serum samples involving cell clotting may be incompatible with this kit. For best results, use serum separators or filtration when preparing serum samples. To ensure proper ionic strength, desalt samples using a desalting column or dialyze samples vs. a low-salt buffer (25mM Tris, 75mM NaCl; pH 7.5 for human and swine albumin) (25mM Tris, 25mM NaCl; pH 7.5 for bovine and calf albumin).
- The Binding/Wash Buffer of 25mM Tris, 75mM NaCl; pH 7.5 provided in the kit has been optimized for binding HSA and reduced binding of non-albumin proteins. For other albumin species such as bovine, calf, rat or goat, an alternative low-salt Binding/Wash Buffer of 25mM Tris, 25mM NaCl; pH 7.5 must be used in all steps. Some species of albumin may not bind to Pierce Albumin Depletion Resin.
- Differing sample types contain a wide range of albumin concentrations. Take care not to exceed the resin's binding capacity. Each 200µL aliquot of Pierce Albumin Depletion Resin binds 2.0mg of HSA.
- Because of the high albumin concentration present in serum, each Pierce Albumin Depletion Resin aliquot of 200µL settled resin can bind sufficient albumin to process only 5-50µL of serum sample. To process larger serum samples volumes, use more resin volume or multiple spin columns. For serum samples containing high albumin concentrations (e.g., > 50mg/mL), dilute sample with the appropriate Binding/Wash Buffer (as described above). For best results, optimize sample conditions for each specific application.

Additional Materials Required

- 1.5-2.0mL microcentrifuge tubes for sample collection

Procedure for Albumin Depletion Using Centrifugation

The following protocol is an example application for this product. Specific applications may require optimization.

Note: Each 200µL aliquot of Pierce Albumin Depletion Resin binds 2.0mg of HSA. Binding capacity can be increased by doubling volume measurements in the procedure below.

1. Shake the resin bottle to resuspend the resin. Using a wide-bore micropipette tip, transfer 400µL of the slurry (corresponding to 200µL settled resin volume) into a spin column and loosely cap the column.

   Note: The amount of resin to use depends on the volume and albumin concentration of the sample being processed.

2. Twist off bottom closure of the spin column and place spin column into a 1.5 to 2.0mL collection tube. Centrifuge at ~12,000 × *g* for 1 minute to remove excess liquid. Discard flow-through and place spin column back into the same collection tube.

   Note: Do not snap off bottom. To remove, twist slightly in one direction followed by the other direction.

3. Add 200µL of Binding/Wash Buffer to the spin column.
4. Centrifuge at 12,000 × *g* for 1 minute. Discard flow-through and place spin column into a new collection tube.
5. Apply 5-50µL of albumin-containing sample to resin and incubate for 1-2 minutes at room temperature.

   Note: Sample must have < 100mM salt for proper albumin binding. Dialyze or dilute sample as needed (see Important Product Information Section concerning sample preparation).

6. Centrifuge at 12,000 × *g* for 1 minute. Re-apply flow-through to spin column and incubate for 1-2 minutes at room temperature to ensure maximal albumin binding.
7. Centrifuge at 12,000 × *g* for 1 minute. Retain flow-through. Place spin column in a new collection tube.
8. Wash resin to release unbound proteins by adding 50µL of Binding/Wash Buffer for each 200µL of resin used. Use the appropriate Binding/Wash Buffer for your albumin type (see Important Product Information Section).
9. Centrifuge at 12,000 × *g* for 1 minute. Retain flow-through. Place spin column in a new collection tube.
10. Repeat Steps 8 and 9 three to four additional times.

    Note: Once the procedure is optimized for a particular application, the wash steps can be increased in volume and reduced in number to simplify sample processing.

11. Analyze retained fractions by SDS-PAGE analysis or by protein concentration determination. Combine desired fractions. Concentrate albumin-depleted sample as needed for further processing.

    Note: For 2D PAGE or mass spectrometry analysis, albumin-depleted samples must be precipitated, dialyzed or desalted to remove interfering salts (see Related Thermo Scientific Products Section).

Optional Elution of Albumin from Resin

1. To elute bound albumin, wash the resin with 200µL of 20mM sodium phosphate, 250mM sodium thiocyanate; pH 7.2.
2. Centrifuge at ~12,000 × *g* for 1 minute. Retain flow-through. Place spin column in a new collection tube.
3. Repeat Steps 1 and 2 three to four additional times.
4. Analyze retained fractions by SDS-PAGE or protein concentration determination. Discard the used spin column.

   Note: Stepwise elution of albumin and other bound proteins using NaCl concentrations between 300mM and 1.5M can be used in place of the 20mM sodium phosphate, 250mM sodium thiocyanate; pH 7.2 elution buffer.

Troubleshooting

Problem	Possible Cause	Solution
High residual albumin in sample	Albumin concentration exceeded resin binding capacity	Reduce sample amount loaded
		Increase resin amount per column
	Salt concentration in sample was too high	Dialyze sample before use or dilute with low ionic strength buffer
	Incompatible serum preparation method was used	Dialyze sample before use with low ionic strength buffer
		Prepare serum by alternative method such as filtration
	Salt concentration in Binding Buffer is too high for specific albumin type	Use low salt (e.g., < 25mM NaCl) or no salt 25mM Tris, pH 7.5 for Binding/Wash Buffer **Note:** This may increase nonspecific protein binding
	Non-compatible albumin type	Ensure albumin type is compatible with the depletion kit

Related Thermo Scientific Products

69705 **Pierce Spin Columns – Screw Cap,** 25 units

89877 **Zeba™ Micro Spin Desalting Columns, 7K MWCO,** 75µL

89882 **Zeba Spin Desalting Columns, 7K MWCO,** 0.5mL

88512 **Protein Concentrators PES, 3K MWCO,** 0.5mL

88400 **Slide-A-Lyzer™ MINI Dialysis Device, 3.5K MWCO,** 0.5mL

24615 **Imperial™ Protein Stain,** 1L

24590 **GelCode™ Blue Stain Reagent,** 500mL

23227 **Pierce BCA Protein Assay Kit**

23235 **Micro BCA Assay Reagent Kit**

22662 **Pierce 660nm Protein Assay Kit**

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

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