Pierce™ Albumin Depletion Kit

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WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from thermofisher.com/support.

Product description

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 200 µL settled resin can process 5–50 µL 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 human serum albumin (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.

Contents and storage

The Pierce™ Albumin Depletion Kit contains sufficient resin and buffer for 24 spin columns.

Item	Amount	Composition	Storage
Pierce™ Albumin Depletion Resin	10 mL	Supplied as 50% resin slurry Binding capacity: 2.0 mg of HSA for 200 µL of settled resin	4°C
Binding/Wash Buffer	11 mL	25 mM Tris, 75 mM NaCl, pH 7.5	
Pierce™ Spin Columns	24 columns	—	

Additional 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 (25 mM Tris, 75 mM NaCl, pH 7.5 for human and swine albumin; 25 mM Tris, 25 mM NaCl, pH 7.5 for bovine and calf albumin).
- The Binding/Wash Buffer of 25 mM Tris, 75 mM 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 25 mM Tris, 25 mM 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 200 µL aliquot of Pierce™ Albumin Depletion Resin binds 2.0 mg of HSA.
- Because of the high albumin concentration present in serum, each Pierce™ Albumin Depletion Resin aliquot of 200 µL settled resin can bind sufficient albumin to process only 5–50 µL 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., >50 mg/mL), dilute sample with the appropriate Binding/Wash Buffer. For best results, optimize sample conditions for each specific application.

Required materials not supplied

- 1.5–2.0-mL microcentrifuge tubes for sample collection

Deplete albumin using centrifugation

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

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

1. Shake the resin bottle to resuspend the resin. Using a wide-bore micropipette tip, transfer 400 µL of the slurry (corresponding to 200 µL 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 the bottom closure of the spin column. Place the spin column into a 1.5–2.0-mL collection tube. Centrifuge at approximately 12,000 x *g* for 1 minute to remove excess liquid. Discard the flow-through and place the spin column back into the same collection tube.

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

3. Add 200 µL of Binding/Wash Buffer to the spin column.

4. Centrifuge at 12,000 x *g* for 1 minute. Discard the flow-through and place the spin column into a new collection tube.

5. Apply 5–50 µL of albumin-containing sample to the resin and incubate for 1–2 minutes at room temperature.

   Note: The sample must have <100 mM salt for proper albumin binding. Dialyze or dilute the sample as needed (see “Additional information” on page 1).

6. Centrifuge at 12,000 x *g* for 1 minute. Re-apply the flow-through to the spin column and incubate for 1–2 minutes at room temperature to ensure maximal albumin binding.

7. Centrifuge at 12,000 x *g* for 1 minute. Retain the flow-through. Place the spin column in a new collection tube.

8. Wash the resin to release unbound proteins by adding 50 µL of Binding/Wash Buffer for each 20 µL of resin used. Use the appropriate Binding/Wash Buffer for your albumin type (see “Additional information” on page 1).

9. Centrifuge at 12,000 x *g* for 1 minute. Retain the flow-through. Place the spin column in a new collection tube.

10. Repeat step 8 and step 9 3–4 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 the retained fractions by SDS-PAGE analysis or by protein concentration determination. Combine the desired fractions. Concentrate the 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.

(Optional) Elute albumin from resin

1. To elute bound albumin, wash the resin with 200 µL of 20 mM sodium phosphate, 250 mM sodium thiocyanate, pH 7.2.

2. Centrifuge at approximately 12,000 x *g* for 1 minute. Retain the flow-through. Place the spin column in a new collection tube.

3. Repeat step 1 and step 2 3–4 additional times.

4. Analyze the 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 300 mM and 1.5 M can be used in place of the 20 mM sodium phosphate, 250 mM sodium thiocyanate, pH 7.2 elution buffer.

Troubleshooting

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

Limited product warranty

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For descriptions of symbols on product labels or product documents, go to thermofisher.com/symbols-definition.

Revision history: Pub. No. MAN0011862

Revision	Date	Description
B.0	7 December 2022	The content and format were updated.
A.0	17 October 2015	New document for the Pierce™ Albumin Depletion Kit.