INSTRUCTIONS

SwellGel® Blue Albumin Removal Kit

PIERCE Biotechnology
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89845 89846

1315.1

Number	Description
89845	**SwellGel® Blue Albumin Removal Kit,** contains sufficient reagents for 12-25 reactions **Kit Contents:** **SwellGel® Blue Albumin Removal Discs*,** 25 discs **Binding/Wash Buffer,** 6.25 ml **Mini-Spin Columns,** 25 columns
89846	**SwellGel® Blue Albumin Removal Kit,** contains sufficient reagents for 50-100 reactions **Kit Contents:** **SwellGel® Blue Albumin Removal Discs*,** 100 discs **Binding/Wash Buffer,** 25 ml **Mini-Spin Columns,** 50 columns

Storage: Upon receipt store at room temperature. Product is shipped at ambient temperature.

This product is guaranteed for one year from the date of purchase when handled and stored properly.

*Patent pending

Introduction

SwellGel® Blue Albumin Removal Discs are designed to aid serum component studies by rapidly removing excess albumin from serum samples. The SwellGel® Blue Discs are composed of a dehydrated support that hydrates rapidly (in less than 20 seconds) when water is added. The hydrated support is a buffer equilibrated Cibacron® Blue-based gel slurry optimized to remove a variety of species-specific albumins. Each SwellGel® Disc is equivalent to 200 µl of resin and offers a binding capacity for human serum albumin of >2.0 mg.

SwellGel® Blue Discs can process 10-150 µl of serum sample in a single reaction. The sample handling process can be performed in less than 10 minutes by low-speed centrifugation or syringe-based methods. Features of this novel technology include exceptional albumin removal capabilities, ease of handling, speed, versatility, convenience, consistency, greater stability of chromatography media and room temperature shipping and storage. SwellGel® Blue Albumin Removal Support has been optimized for human serum albumin but will also bind to swine and sheep serum albumin effectively. Furthermore, with a slight modification of the binding buffer, SwellGel® Blue Albumin Removal Discs will remove bovine, calf, goat and rat albumin. This product, however, is not recommended for mouse albumin.

Abbreviated Procedure

Total procedure time: 10 minutes

1. Prepare resin.
2. Bind samples containing albumin.
3. Wash 1-4 times to release albumin-free sample.

Important Product Information

- Use a clarified (centrifuged or filtered) protein solution to ensure proper resin flow.
- This kit has been developed and optimized for binding human serum albumin, with the Binding/Wash Buffer formulated to reduce binding of non-albumin proteins. However, this formulation prevents the potential binding of many other albumin types. For albumin species such as bovine, calf, rat or goat, this product can be effective if the sample is free of excess salt (<50 mM) and an alternative Binding/Wash buffer of 25 mM Tris pH 7.2 is used in all steps. SwellGel® Blue Discs are not guaranteed to function with all species of albumin.
- Albumin Binding will occur at a pH range of 6.0-9.0; however, samples must be free of excess salt. Therefore, methods for preparing serum samples involving cell clotting may not be compatible with this kit. For best results, use serum separators or filtration when preparing serum samples. To ensure proper ionic strength, samples may be passed through a desalting column or dialyzed 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).
- Differing sample types contain a wide range of albumin concentrations. Therefore, take care not to exceed the resin's binding capacity. Each SwellGel® Blue Disc binds >2 mg HSA.
- Because of the high albumin concentration present in serum, typically each SwellGel® Blue Disc can only bind sufficient albumin to process 5-50 µl of serum sample. However, load volumes of at least 50 µl are required to ensure proper resin wetting. For serum samples containing a high albumin concentration, dilute sample to ≥ 50 µl with the appropriate Binding/Wash buffer (as described above). For dilute samples, volumes ≥50 µl can be applied to column. For best results, optimize sample conditions for each specific application.
- Store at room temperature. Keep container tightly capped. Do not allow moisture to enter the container. Do not freeze.

Additional Materials Required

- 1.5-2.0 ml microcentrifuge tubes for sample collection

Procedure for Albumin Removal Using Centrifugation

The following protocol is an example of an application for this product. Your specific application may require optimization.

Note: SwellGel® Blue Albumin Removal Discs are not compatible with all forms of albumin.

Note: Each SwellGel® Blue Albumin Removal Disc will bind >2 mg human serum albumin.

1. Place 1 or 2 discs into a mini-spin column.

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

2. Hydrate SwellGel® Blue Disc with 380 µl of ultrapure water for each disc used. Discs should fully hydrate in less than 20 seconds. Vortex for 1-2 seconds.
3. Twist off bottom closure of the Mini-Spin Column and loosen cap.

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

4. Place column into a 1.5 to 2.0 ml collection tube. Centrifuge at ~12,000 x *g* for 1 minute in a microcentrifuge tube to remove excess liquid. Discard flow-through.
5. Load 50-100 µl of albumin-containing sample for each disc used. **See Important Product Information section concerning sample preparation.**

   Note: Sample must be free of excess salts (<150 mM) for proper albumin binding. Dialyze or dilute sample as needed. See Important Product Information concerning sample preparation.

6. Incubate for 1-2 minutes.
7. Centrifuge at ~12,000 *x g* for 1 minute. Recover flow-through and re-apply to column.
8. Incubate for 1-2 minutes to ensure maximal albumin binding.

9. Centrifuge at ~12,000 *x g* for 1 minute. Retain flow-through. Place column in a new collection tube.
10. Wash resin to release unbound proteins by adding 50 µl Binding/Wash Buffer for each disc used.

    Note: Use the appropriate Binding/Wash Buffer for your albumin type. See Important Product information section.
11. Centrifuge at ~12,000 *x g* for 1 minute. Retain flow-through. Place column in a new collection tube.
12. Repeat Steps 9 and 10 three to four additional times.
13. Analyze retained fractions by SDS-PAGE analysis or by protein concentration determination.
14. Combine desired fractions. Concentrate albumin-free sample as needed for further processing.

    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.

Optional removal of albumin from resin

1. Elute bound albumin by washing the resin with 200 µl 0.25 M sodium thiocyanate, 0.02 M sodium phosphate, pH 7.2.
2. Centrifuge at ~12,000 *x g* for 1 minute. Retain flow-through. Place 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.
5. Dispose of column; do not reuse.

   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 0.2 M sodium thiocyanate, 0.02 M sodium phosphate, pH 7.2 elution buffer.

Troubleshooting

Problem	Cause	Solution
High residual albumin in sample	Albumin concentration exceeds resin binding capacity	Reduce sample amount loaded
	Salt concentration in sample is too high	Dialyze sample before use or dilute with low ionic strength buffer
	Non-compatible serum preparation method 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 20 mM phosphate buffer, pH 7.5 for binding and wash buffer **Note:** This may increase nonspecific protein binding
	Non-compatible albumin type	None

Related Pierce Products

69705	**Handee™ Mini-Spin Columns Plus Accessories,** 25 units
66373	**Slide-A-Lyzer® Dialysis Cassettes,** 7K MWCO, 0.1-0.5 ml capacity
24590	**GelCode® Blue Stain Reagent,** 500 ml, sufficient for staining up to 25 SDS-PAGE 8 cm x 10 cm gels
24602	**GelCode® SilverSNAP® Stain Kit,** sufficient for staining up to 20 SDS-PAGE 8 cm x 10 cm gels
23236	**Coomassie® Plus Protein Assay Reagent**
23227	**BCA Protein Assay Kit**
23235	**Micro BCA™ Assay Reagent Kit**
23240	**Modified Lowry Protein Assay Reagent** Luer-Lok® is a trademark of Becton Dickinson and Company

Cibacron® Blue is a trademark of Ciba Geigy

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