AlbuTrial Kit™ - AlbuVoid™ & AlbuSorb™

AlbuTrial kit™ includes 1 gm of AlbuSorb™ and 5 Preps of AlbuVoid™ with respective buffers.

Proteomic analysis of serum and the quantification of serum proteins as disease markers have often been hampered by the predominance of albumin. BSG offers two different strategies for albumin depletion. Both AlbuSorb™ and AlbuVoid™ were tested on human, sheep, bovine, mouse, goat, rat, and calf from serum and plasma using a simple spin-filter format. AlbuSorb™ facilitates binding of albumin (>90%) with recovery of the remaining serum proteins in the flow-through. AlbuVoid™ voids out or negatively selects the majority of albumin (>90%) in the flow-through and wash fractions. In the elution step the albumin depleted serum proteins can be recovered.

AlbuVoid™

Albumin Depletion Plus Low Abundance Serum/Plasma Protein Enrichment

- Albumin voids in flow-through >95%, with <30 minute bind/wash/elute protocol
- Low abundance enrichment equivalent or better than hexa-peptides or immuno-affinity
- Disposable, cost-effective, no column regeneration or cross-contamination
- Mild elution maintains tertiary structure and simple transfer to secondary analysis
- The eluted fractions retain their enzymatic and biological activity
- Works for all species tested including human, sheep, bovine, goat, rat, mouse, and calf.
- No molecular weight or pI bias
- On-bead protocols improve workflow and efficiency

AlbuVoid™ is an albumin depletion reagent kit. It removes albumin from serum and plasma samples while concentrating low abundance proteins. The **AlbuVoid™** protocol uses mild buffers; the protocol conditions are so gentle that native enzyme activity is retained in elution fractions.

AlbuVoid™ does not bind albumin. The low abundance proteins which bind to **AlbuVoid™**, are eluted off without the albumin. Consequently, the low abundance serum proteins are enriched. It is ideal for applications involving discovery and targeted proteomics, enzyme assays, toxicological studies for new drugs, protein profiling, protein arrays, 1D and 2D gel electrophoresis, LC-MS, and cytokines research.

AlbuVoid™ derives from a silica-based library of individual mixed-mode polymeric ligands. The library was designed to facilitate weak binding of proteins, allowing for rapid elution from the matrix without any foreknowledge of the variety of proteins contained in the starting sample. Because of its specialized voiding properties, **AlbuVoid™** depletes high abundance proteins in serum like albumin while improving the resolution of less abundant serum proteins.

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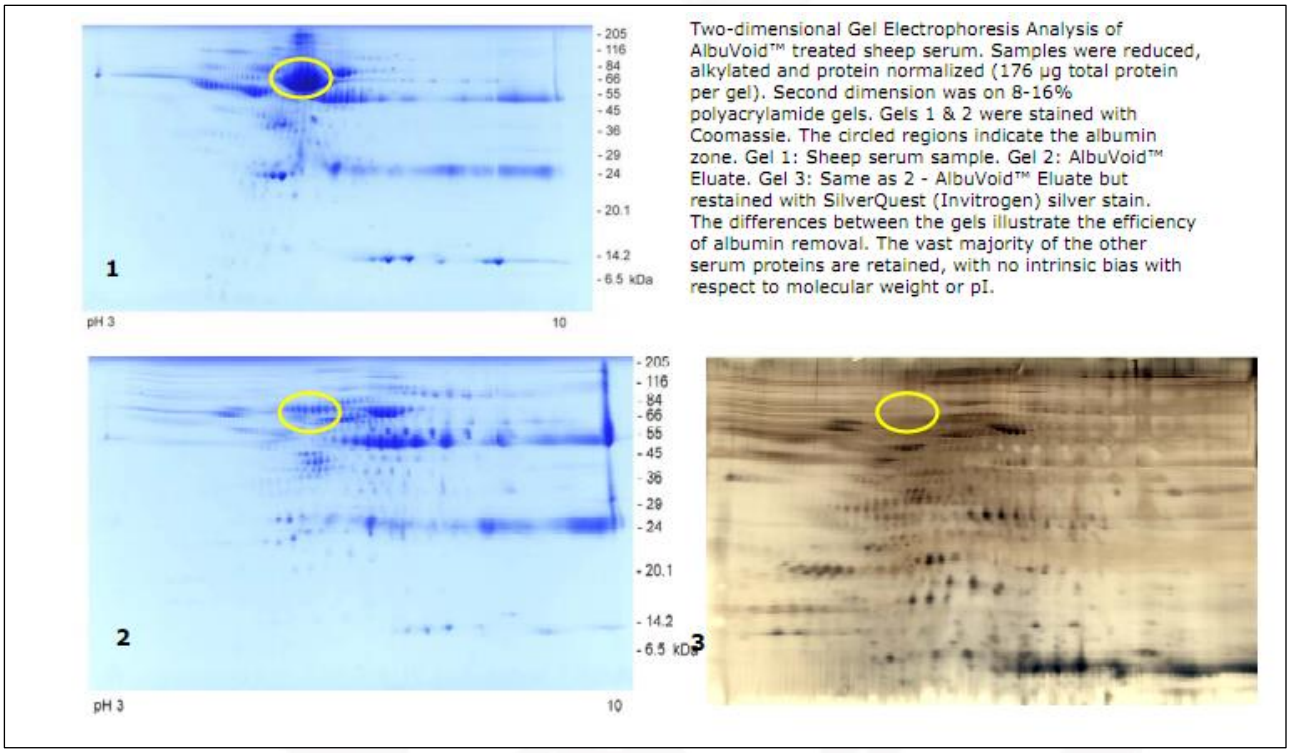

Two-dimensional Gel Electrophoresis Analysis of AlbuVoid™ treated sheep serum. Samples were reduced, alkylated and protein normalized (176 µg total protein per gel). Second dimension was on 8-16% polyacrylamide gels. Gels 1 & 2 were stained with Coomassie. The circled regions indicate the albumin zone. Gel 1: Sheep serum sample. Gel 2: AlbuVoid™ Eluate. Gel 3: Same as 2 - AlbuVoid™ Eluate but restained with SilverQuest (Invitrogen) silver stain. The differences between the gels illustrate the efficiency of albumin removal. The vast majority of the other serum proteins are retained, with no intrinsic bias with respect to molecular weight or pI.

AlbuSorb™

Albumin Depletion From Serum or Plasma

- AlbuSorb™ binds approximately 30 mg albumin/ml and serum proteins flow through.
- Affinity-type equivalence, virtually no cross-reactivity with other proteins
- Disposable, cost-effective and no cross-contamination
- Economical new surface technology, not based on affinity chromatography
- Mild condition maintains tertiary structure and simple transfer to secondary analysis
- The albumin depleted flow through fractions retain their enzymatic and biological activity
- Removes >90% albumin from many species including human, sheep, bovine, mouse, goat, rat, and calf from serum and plasma.

Poly-electrolytes are polymers with repeating units of stationary charges. AlbuSorb™ comes from a class of solid-phase, or surface-based, elastomeric poly-electrolytic surfaces that bind proteins through an empirically derived chemistry combining elements of polymer composition, cross-linking architecture and charge properties. As with bio-polymers like DNA and Heparin, governing their reactivity is the spatial presentation of the electrostatic groups along a flexible polymer chain. This same strategy was used in the creation of both Viraffinity™ and HemogloBind™.

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Unlike immuno-affinity, the surfaces utilized are disposable eliminating cycle to cycle variance and cross-contamination. AlbuSorb™ is supplied as a powder. Simply weigh, centrifuge and/or filter, and recover the albumin depleted serum in the supernatant.

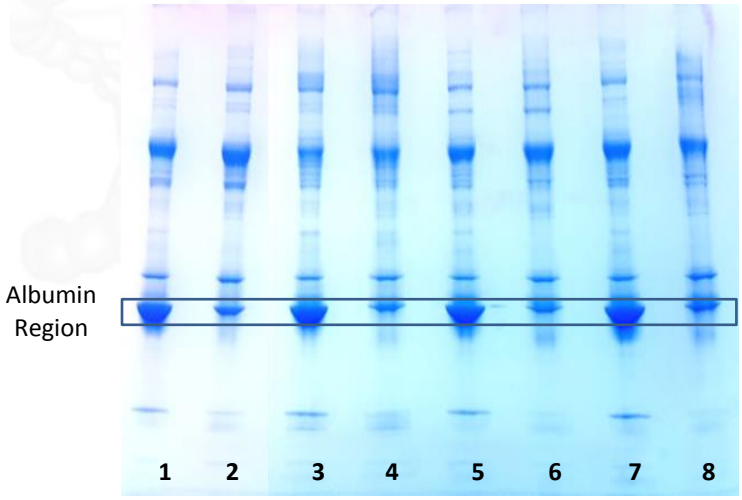
Cancer Sera Before and After AlbuSorb™

1: Normal pooled serum control
2: Flow-through from normal serum

3: Breast cancer pooled serum control
4: Flow-through from breast cancer serum

5: Lung cancer pooled serum control
6: Flow-through from lung cancer serum

7: Pancreatic cancer pooled serum control
8: Flow-through from pancreatic cancer serum
Note: All samples are from human female ages 40-60



Gel Image: SDS-PAGE non-reduced, Criterion™ Tris.HCl (Bio-Rad) 4-15%

AlbuTrial Kit™ AVS-05 Includes

AlbuVoid™ Kit			AlbuSorb™ Kit	
Reagent (For 5 preps)	**Quantity**		**Reagent (For 28 preps)**	**Quantity**
AlbuVoid™ AVK-5	0.25 gram		AlbuSorb™ A185-1	1 gram
Binding Buffer AVBB	4 ml		Binding Buffer BB1	30ml
Wash Buffer AVWB	5 ml			
Elution Buffer AVEB	2 ml			
Corning® Spin-X Filter	5			

Product	Size	Quantity of Serum Processed	Item No.
AlbuTrial Kit™	5 Preps AlbuVoid™ and 1 gram of AlbuSorb™	1 gram of AlbuSorb™ processes 20 preps (25 µl serum) and 5 Preps of AlbuVoid™ (processes 200 µl serum)	AVS-05

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AlbuVoid™

PROTOCOL – Based on processing 100-200 µl Serum or Plasma

For best results – the lysate should be clear and free of colloidal material. We recommend first filtering through a 0.45 µm syringe-type filter before beginning the prep.

1. Weigh out 50 mg of **AlbuVoid™** matrix in a spin-tube (0.45µ SpinX centrifuge tube filter from Corning).

2.Add 250 µl of **Binding Buffer AVBB .**Vortex for 5 minutes at room temperature followed by centrifugation at 3000 rpm. Discard the supernatant.

3.Repeat step-2

4.Condition by adding 200 µl of **AVBB** and 200 µl of the **Serum.** Vortex for 10 min and then centrifuge for 4 minutes at 10,000 rpm.

5. Remove the albumin enriched supernatant (Flow-Through) **FT**.

6. To the pellet add 500 µl of **Wash Buffer AVWB.** Vortex for 5 min and centrifuge for 4 minutes at 10,000 rpm. Remove the soup as **Wash.**

7. Repeat Step-6.

8. To the pellet add 400 µl of **Elution Buffer AVEB.** Vortex for 10 min and centrifuge for 4 minutes at 10,000 rpm. Remove the filtrate as elution (albumin depleted proteins).

Note:

- **Download related product AlbuVoid™ LC-MS On-Bead Trypsin Digestion Protocol**
- The protocol can be scaled up or down proportionally to adjust for different serum volumes. The surface amount can be adjusted to accommodate more or less albumin removal.
- We have 0.45µ SpinX centrifuge tube filters. If required can be ordered separately.

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AlbuSorb™

PROTOCOL – Based on processing 25 µl Serum or Plasma

For best results – the lysate should be clear and free of colloidal material. We recommend first filtering through a 0.45 µm syringe-type filter before beginning the prep.

1. Weigh out 50 mg of AlbuSorb™ Powder in a spin-tube.

2. Add 400 µl of **Binding Buffer BB1** to condition the AlbuSorb™ powder. Shake it manually/ vortex for 3 min and then centrifuge for 2 minutes at 3000 rpm. Discard the supernatant.

3. Repeat step-2

4. As a requirement for albumin binding, add 250 µl of the **BB1 Buffer** and then add 25 µl of the serum to **Step 3**. Mix for 10 minutes on a rotating shaker.

5. Centrifuge for 2 minutes at 3000 rpm, **supernatant (flowthrough) contains serum proteins minus albumin.**

6. Optionally the pellet **(mostly albumin)** can be eluted with 200 µl of **stripping buffer (0.2M Tris + 0.5M NaCl pH9.5 by mixing on a shaker for 10 min)** and centrifuge for 2 minutes at 3000 rpm.

The protocol can be scaled up or down proportionally to adjust for different serum volumes. The surface amount can be adjusted to accommodate more or less albumin removal.

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