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AlbuVoid™ Albumin Depletion Kit

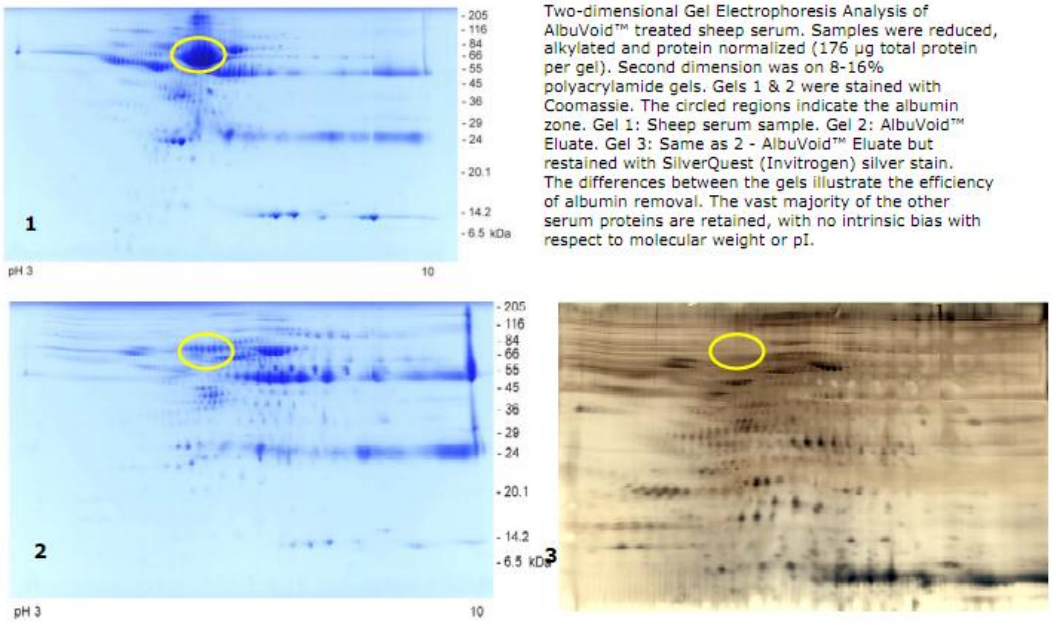
Albumin Depletion Plus Low Abundance Serum Protein Enrichment

- Albumin voids in flow-through >95%, with <30 minute bind/wash/elute protocol
- Low abundance enrichment equivalent or better than hexa-peptides or antibodies
- 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
- Removes albumin from many species including human, sheep, bovine, goat, rat, and calf.

AlbuVoid™ is a albumin depletion reagent kit. It removes albumin from serum and plasma samples while concentrating low abundance, and/or low molecular weight proteins. The AlbuVoid™ protocol uses mild buffers; the protocol conditions are so gentle that native enzyme activity is retained in elution fractions. AlbuVoid™ considerably enhances resolution of proteins below 50 kD, a limitation of alternate enrichment protocols.

AlbuVoid™ does not bind albumin. All other proteins(except albumin) in the sample binds to AlbuVoid™ and then you can elute off all the proteins minus the albumin. Resulting in low abundance serum protein enrichment. It is ideal for applications involving biomarker discovery, enzyme assays, toxicological studies for new drugs, protein profiling using SELDI analysis, protein array pixelation ,1D and 2D gel electrophoresis, LC/MS, and MALDI-TOF 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 specific binding properties, AlbuVoid™ depletes high abundance proteins in serum like albumin while improving the resolution of less abundant serum proteins.



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.

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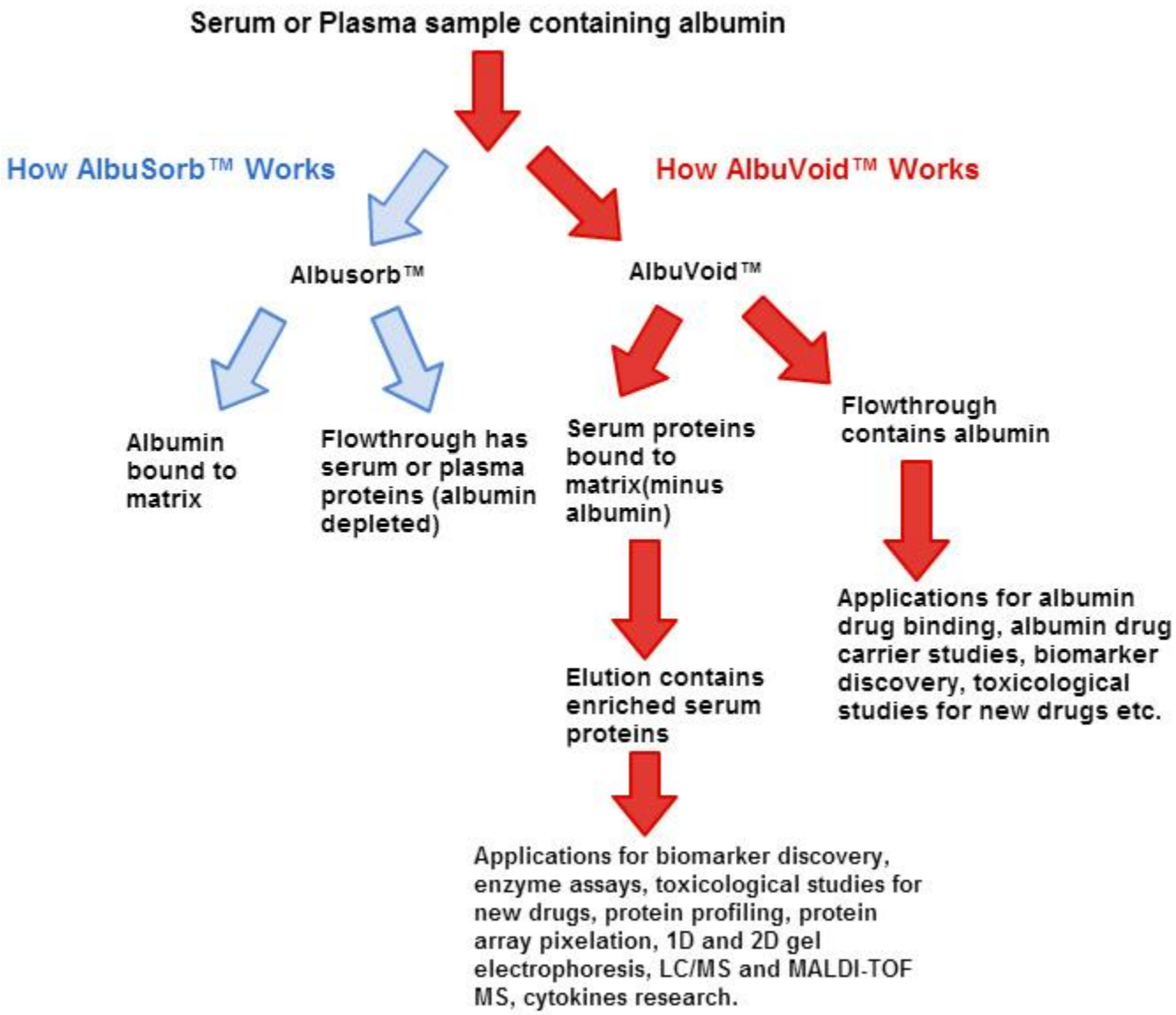
Depletion of albumin using Albusorb™ or Albuvoid™



Product	Size	Total samples processed	Item No.	Price
AlbuVoid™	5 Preps	1ml of sample	AVK-05	$225
AlbuVoid™	10 Preps	2ml of sample	AVK-10	$310
AlbuVoid™	50 Preps	10 ml of sample	AVK-50	$850
Note: Please contact sales@biotechsupportgroup.com for prices in bulk amount.				

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Items Required	5 Prep	10 Prep	50 Prep	Reagent
AlbuVoid™	0.25 gram	0.5 gram	2.5 grams	Supplied
Binding Buffer AVBB, *PH 6.0*	6 ml	12 ml	60 ml	Supplied
Wash Buffer AVWB, *PH 7.0*	6 ml	12 ml	60 ml	Supplied
Elution Buffer AVEB, *PH 9.8*	6 ml	12 ml	60 ml	Supplied
SpinX Centrifuge tube filters	5	10	50	Supplied

PROTOCOL – Based on processing 100-200 µl Serum

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. **The bead is now enriched with albumin depleted proteins. For on-bead digestion for LC-MS work see on-bead digestion protocol, otherwise proceed to the next step.**

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). The eluate is ready for further functional or LC-MS studies.

Note:

- 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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Suggested On-Bead Digestion Protocol

- After the final wash steps from step 7, add 100 µls of 5 mM DTT solution to the beads for complete immersion, mix and incubate at 60˚C for ½ hour.
- After cooling, add 100 µls of 25 mM iodoacetamide to the DTT/bead suspension, mix and incubate in the dark for 1 hour.
- Centrifuge at 5000xg (medium setting, not max) for 3 mins, and discard supernatant. Transfer the filter slurry of beads, DTT and iodoacetamide to a clean Eppendorf tube.
- On-bead digestion is done by adding 100 µls of a 0.025 ug/uL solution of MS-grade. Trypsin to the beads. Digest overnight at 37˚C.
- Centrifuge at 5000xg (medium setting, not max) for 3 mins, and retain peptide filtrate.
- To further extract remaining peptides, add 100 µls of 10% solution of formic acid to the beads.
- Incubate for 15 minutes at 37˚C, centrifuge at 5000xg (medium setting, not max) for 3 mins, and add this volume to the first volume.
- Reduce to a final volume of 100 µls using a SpeedVac and store at -80 ˚C until LC-MS/MS.

References:

Serum

Serum Profiling Making Mark on Predictive Medicine.Vicki Glaser.Genetic Engineering & Biotechnology News. 2011;31(7):1-55.

Patent

Narain, Niven Rajin, Rangaprasad Sarangarajan, and Vivek K. Vishnudas. "INTERROGATORY CELL-BASED ASSAYS AND USES THEREOF." U.S. Patent No. 20,120,258,874. 11 Oct. 2012.

CONTACT US

We welcome your questions and comments regarding our products.

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