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AlbuTrial Kit™ - AlbuVoid™ & AlbuSorb™

AlbuTrial kit™ includes 1 gm of AlbuSorb™ and 5 Preps of AlbuVoid™ with respective buffers. Matrix facilitating effective removal of albumin from serum. Proteomic analysis of serum and the quest for identifying serum proteins as disease markers have often been hampered by the predominance of proteins like albumin. The unusually high abundance of albumin in serum represses the signals for low abundance proteins, thereby interfering with the resolution and sensitivity of many protein profiling techniques. In the course of evaluating several available methods and commercial kits, we have been able to refine the albumin depletion protocols and establish a modified albumin removal method using ligand based protein fractionation system. The matrices AlbuSorb™ and AlbuVoid™ were tested on human, sheep, bovine, mouse, goat, rat, and calf from serum and plasma using a simple protocol. The procedure is pretty simple, just add, centrifuge and/or filter, and recover the albumin depleted serum. AlbuSorb™ facilitates binding of albumin (>90%) thereby removing rest of the proteins in the flow-through. AlbuVoid™, on the other hand, flushes 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 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, mouse, sheep, bovine, goat, rat, and calf from serum and plasma.

AlbuVoid™, a silica-based protein enrichment matrix, 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™ derives from a silica-based library of individual mixed-mode ligand combinations (ionic, hydrophobic, aromatic, polymer). 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. In contrast to traditional chromatographic methods, our weak binding approach is more selective, presumably because of a lower degree of non-specific protein-protein interactions at the surface interface. In the case of AlbuVoid™, a single, mixed-mode ligand architecture was selected empirically from the library. Because of its specific binding properties, AlbuVoid™ depletes high abundance proteins in serum like albumin and immunoglobulins while improving the resolution of less abundant serum proteins.

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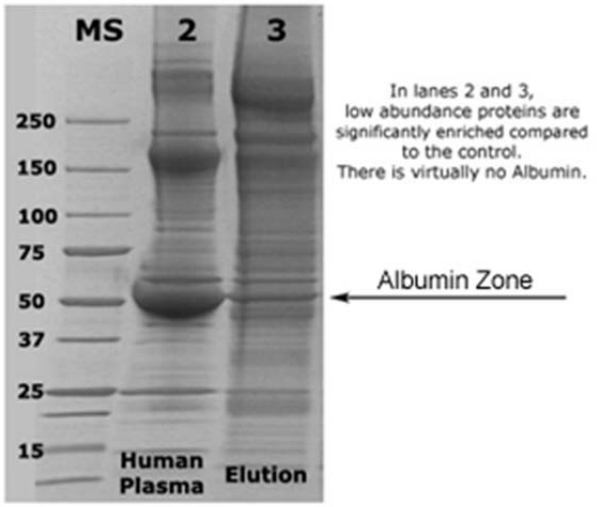


AlbuVoid™ - Albumin Depletion Kit from Serum or Plasma image 2

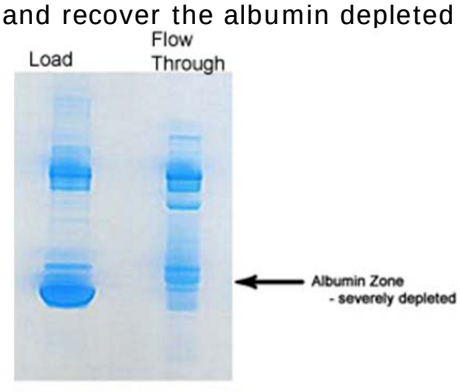
The AlbuVoid™ matrix increases the dynamic range of protein detection. Fifty micrograms of protein were loaded per lane. **Lane 2** represents untreated plasma. **Lane 3** designates the albumin depleted plasma zone. **SDS-PAGE:** Samples were loaded onto pre-cast 4-20% Tris-HCl gels with 1X Tris-glycine SDS running buffer (Bio-Rad). As a reference **MS** is 15µl of Precision Plus™ Unstained Standards (Bio-Rad). Gels were fixed in a 25% isopropanol, 10% acetic acid solution and stained with Bio-Rad 0.025% CBB R250, 25% isopropanol, and 10% acetic acid staining solution. All gels were destained in multiple changes of distilled water.

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. 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.



AlbuSorb™ Albumin Depletion Kit

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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	12 ml		Binding Buffer BB1	30ml
Wash Buffer AVWB	12 ml			
Elution Buffer AVEB	12 ml			
Corning® Spin-X Filter	5			
Note: Please contact sales@biotechsupportgroup.com for prices in bulk amount.				

Product	Size	Quantity of Serum Processed	Item No.	Price
AlbuTrial Kit™	.25 grams of AlbuVoid™ and 1 gram of AlbuSorb™	1 gram of AlbuSorb™ and 5 Preps of AlbuVoid™	AVS-05	$350

AlbuVoid™

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

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).

9. Read at 280nm using a spectrophotometer.

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.

AlbuSorb™

PROTOCOL – Based on processing 25 µl Serum or Plasma

1. Weigh out 35 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.

AlbuSorb™ References

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Plasma
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On-Bead Digestion Protocols For LC-MS Proteomic Workflows
Swapan Roy, Ph.D., Matthew Kuruc, Krishna Patel, Suzanne Ackloo, Sven Nahnsen, Ph.D. On-Bead Digestion Protocols Improve LC-MS Workflows Of Albumin Depleted Samples. US HUPO Conference March 11,12, 2013.

CONTACT US

We welcome your questions and comments regarding our products.

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