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AlbuVoid™

Albumin Depletion Plus Low Abundance Serum Protein Enrichment from Serum, Plasma, Tissues And Culture Media

- 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
- Low abundance protein and enzyme enrichment, suitable for all analytical platforms (i.e., LC-MS, Immunoassay)
- Species agnostic, validated on human, sheep, bovine, goat, fish, etc.
- No molecular weight or pI bias
- For LC-MS, optional seamless On-bead protocols (BASP™) workflows and unique proteolytic efficiencies
    - No in-gel digests, no solution digests, no C18 desalting, more consistent, reproducible results
    - Compatibility with quantitative label (i.e., iTRAQ) and label-free LC-MS methods

AlbuVoid™ is an albumin depletion reagent kit, however the beads do not bind albumin. It removes albumin from serum and plasma samples while enriching low abundance proteins on the beads. The **AlbuVoid™** protocol uses mild buffers; the protocol conditions are very gentle so that native enzyme and functional activity is retained in elution fractions.

AlbuVoid™ beads are derived from a silica-based library of individual imperfect fit polymeric ligands. The library was designed to facilitate weak binding of proteins, allowing for preferential displacement of the stronger bias binding proteins. Finally, **AlbuVoid™** method can deplete Albumin and enrich remaining serum sub-proteome without the use of antibodies.

The low abundance proteins which bind to **AlbuVoid™** beads, 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, immunoassay and microarrays, 1D & 2D gel electrophoresis and LC-MS.

The **AlbuVoid™** beads have been adapted to a protocol specifically designed for LC-MS applications whereby the low abundance proteome adsorbed to the beads is Trypsin degraded to its peptide constituents. This is called Bead Assisted Sample Prep or BASP™; the protocol is included as an optional digest method.

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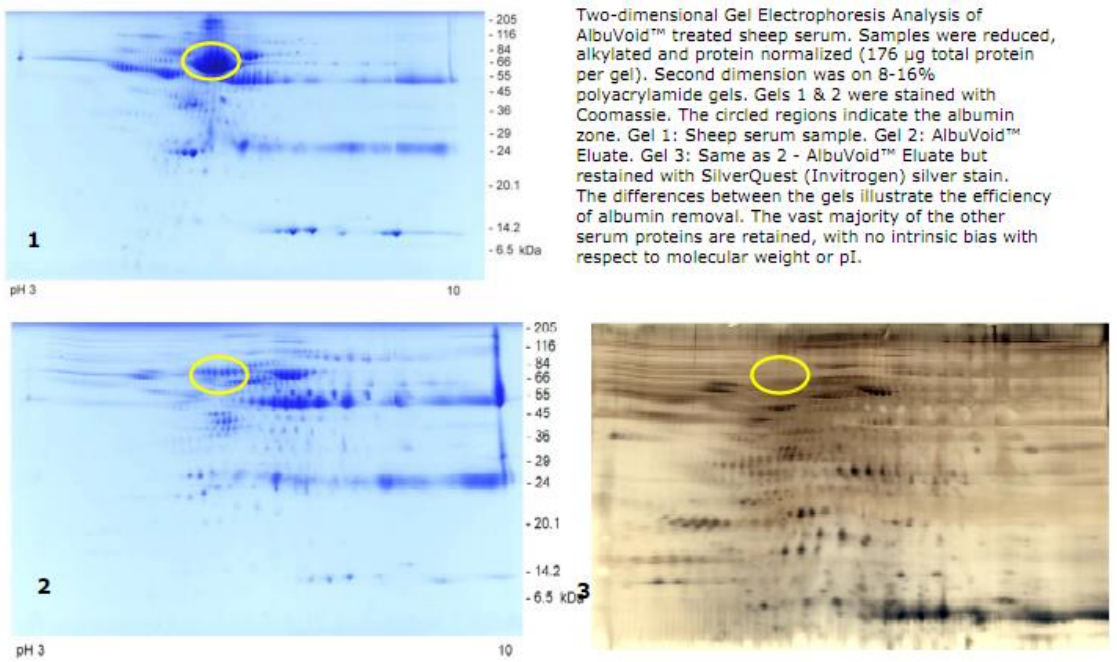
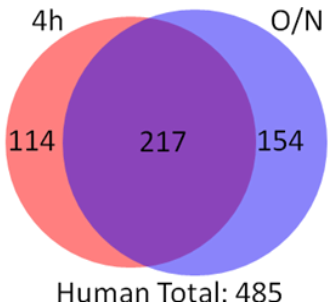


Two-dimensional Gel Electrophoresis Analysis of AlbuVoid™ treated sheep serum. Samples were reduced, alkylated and protein normalized (176 µg total protein per gel). Second dimension was on 8-16% polyacrylamide gels. Gels 1 & 2 were stained with Coomassie. The circled regions indicate the albumin zone. 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.

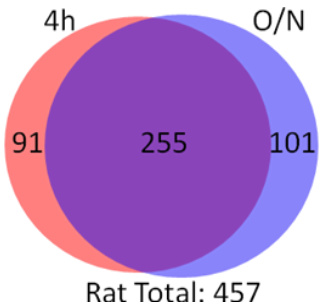
Comparison of 4 hour & Overnight Digestion Times

The total **AlbuVoid™** bound proteins were compared for human and rat sera at two different on-bead (BASP™) digestion times, 4 hours and overnight (O/N). Note that many identified proteins overlap while certain populations of proteins were only observed in one or the other digest time (Zheng et al, J Proteomics Bioinform 8: 225-230, 2015).

Number of unique protein IDs after AlbuVoid™



Human Total: 485



Rat Total: 457

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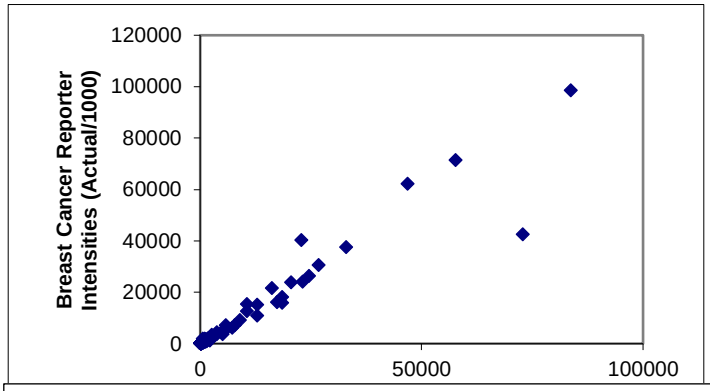
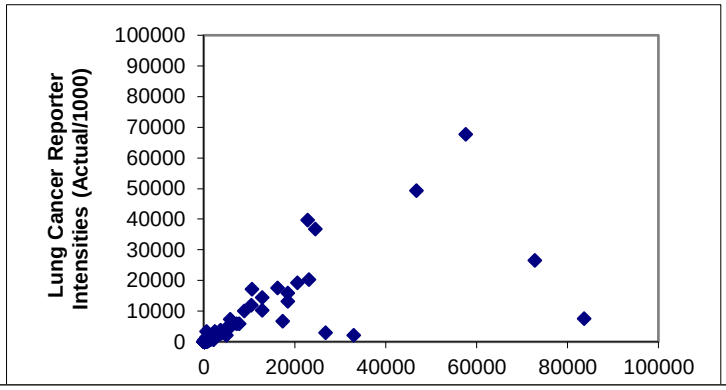
iTRAQ Labeled Peptides From Two Representative Disease Serum Samples

A. Breast Cancer Serum Proteins vs. Normal Serum Proteins.



B. Lung Cancer Serum Proteins vs. Normal Serum Proteins.



The reporter intensity signals were added together for each of the iTRAQ labeled peptides supporting the associated protein identification. Each of the additive peptide reporter intensities were then plotted for each protein comparing the following sample pairs: A. Breast Cancer Serum Proteins vs. Normal Serum Proteins. B. Lung Cancer Serum Proteins vs. Normal Serum Proteins. C. Breast Cancer Serum Proteins vs. Lung Cancer Serum Proteins. The linearity with demonstrates the reproducibility of the AlbuVoid™ with BASP™ digestion method for discovery applications.

Product	Size	Total samples processed	Item No.
AlbuVoid™	5 Preps	5 x 200 µl samples	AVK-05
AlbuVoid™	10 Preps	10 x 200 µl samples	AVK-10
AlbuVoid™	50 Preps	50 x 200 µl samples	AVK-50
Note: Please contact sales@biotechsupportgroup.com for prices in bulk quantities.			

Albumin and IgG Removal Products

AlbuSorb™ PLUS and **AlbuVoid™ PLUS** are products that deplete both Albumin and IgG. For more information, go to:

https://www.biotechsupportgroup.com/AlbuVoid-PLUS-p/np-avk.htm

https://www.biotechsupportgroup.com/AlbuSorb-PLUS-p/apk285.htm

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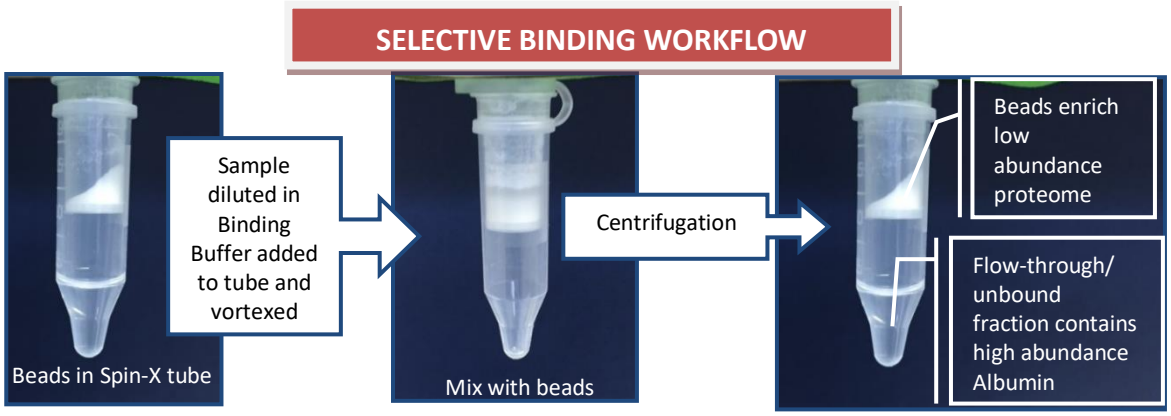
Items Required	5 Prep	10 Prep	50 Prep	Item
AlbuVoid™ Beads	0.25 gram	0.5 gram	2.5 grams	Supplied
Binding Buffer AVBB (0.05M HEPES, pH 6.0)	4 ml	8 ml	40 ml	Supplied
Wash Buffer AVWB (0.05M HEPES, pH 7.0)	5 ml	10 ml	50 ml	Supplied
Elution Buffer AVEB (0.25M Tris + 0.5M NaCl, pH 10.0-10.5)	2 ml	4 ml	20 ml	Supplied
Spin-filter & tube assemblies	5	10	50	Supplied

Additional Spin-Filters (low protein binding, 0.45 µm filter element) can be purchased separately, please inquire.

If there are any questions about compatibility or substitution with other buffers, please contact us.



PROTOCOL – Based on processing 100-200 µl Serum

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.

The centrifugation time may vary, adjust as necessary to get complete filtration through the beads.

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

1. **Bead Conditioning.** Weigh out 50 mg of **AlbuVoid™** beads in a spin-filter. Add 250 µl of **Binding Buffer AVBB.** Vortex or mix thoroughly for 5 minutes at room temperature followed by centrifugation for 2 minutes at 5,000-10,000 rpm (2,000-8,000xg). Discard the filtrate. Repeat step-1.

2. **Sample Processing.** Add 200 µl of **Binding Buffer AVBB** to beads followed by 200 µl of the Serum to the beads. Vortex or mix thoroughly for 10 min and then centrifuge for 4 minutes at 5,000-10,000 rpm (2,000-8,000xg).

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3. Remove the filtrate as Flow-Through, containing the unbound Albumin.

4. To the beads, add 500 µl of **Wash Buffer AVWB.** Vortex for 5 min and centrifuge for 4 minutes at 5,000-10,000 rpm (2,000-8,000xg). Discard the **Wash** filtrate.

5. Repeat Wash Step-4. The **AlbuVoid™** beads contain the enriched Albumin-depleted sub-proteome.

6. **BEAD ELUTION.** To the beads, add 400 µl of **Elution Buffer AVEB.** Vortex for 10 min and centrifuge for 4 minutes at 5,000-10,000 rpm (2,000-8,000xg). Recover the filtrate as elution (albumin depleted proteins). The proteome eluate (0.25M Tris + 0.5M NaCl, pH 10.0-10.5) is ready for further functional, proteomic or LC-MS analysis.

As an option for LC-MS sample preparation, the bead assisted on-bead digestion protocol (BASP™) is provided below. The digest buffer is not supplied but the Wash Buffer (0.05M HEPES, pH 7.0), can be made up, or comparable buffers (0.02-0.5M, pH 6-7) can be used. Higher pH buffers are not recommended.

6. Using **Wash Buffer**, prepare to 10mM of DTT concentration, and add 200 µl to the beads and vortex for 10 minutes and incubate for 30 minutes at 60C.

7. Cool the samples to RT, add suitable volume of Iodoacetamide to 20mM and incubate in the dark for 45 minutes

8. Centrifuge at 5,000-10,000 rpm (2,000-8,000xg) for 5 minutes, and discard filtrate. Rinse the bottoms of the spin-filter tubes with 500 µl of 50% ACN, **Wash Buffer** twice, to remove any traces of the filtrate.

9. Add 16 µg trypsin in 200 µl **Wash Buffer** to the beads and keep at 37°C for a minimum 4 hours to maximum overnight. Overnight is recommended to start with. In select targeted circumstances, 2 hours may be sufficient.

10. Centrifuge at 5,000-10,000 rpm (2,000-8,000xg) for 5 minutes, and retain digested peptides filtrate.

11.To further extract remaining peptides, add 300 µL 10% formic acid, vortex 10 min, centrifuge at 5,000-10,000 rpm (2,000-8,000xg) for 5 mins., and combine this volume with volume from step 10.

12.Total is about 500µl. Prepare to desired final concentration. Store at -80°C until LC-MS/MS.

Selection of AlbuVoid™ Reference Applications:

Serum

Vialaret, Jerome & Kadi, Sarah & Tiers, Laurent & O Flynn, Robin & Lehmann, Sylvain & Hirtz, Christophe. (2018). Albumin depletion of human serum to improve quantitative clinical proteomics. *Current Topics in Peptide & Protein Research* 19. 53-62. http://www.researchtrends.net/tia/abstract.asp?in=0&vn=19&tid=26&aid=6192&pub=2018&type=3

In this work, the investigators focused on depleting albumin from human serum samples using an albumin depletion and low abundance protein enrichment kit – AlbuVoid™, which enabled the detection of several low-abundance proteins. By employing an optimized protocol, enriched proteins known as biomarkers for various diseases were identified. The authors concluded that the **AlbuVoid™ depletion method proved to be faster and more cost-effective than antibody based methods**, and could be helpful for biomarker enrichment and detection in medical research.

Jing, Lun, et al. "PROTEOMIC ANALYSIS IDENTIFIED LBP AND CD14 AS KEY PROTEINS IN BLOOD/BIPHASIC CALCIUM PHOSPHATE MICROPARTICLE INTERACTIONS." *Acta Biomaterialia* (2021). Here, in a LC-MS/MS proteomic study, the article describes use of **HemoVoid™** and **AlbuVoid™** prior to LC-MS analysis, "…After **albumin depletion**, analysis of the significant deregulated proteins showed that **27 signaling pathways significantly changed in blood cells…**"

Poillet-Perez, Laura, et al. "Autophagy maintains tumour growth through circulating arginine."*Nature* (2018): 1.

One *in vivo* model to study autophagy is whole-body deletion of the essential autophagy gene *Atg7* in adult mice which causes a systemic metabolic defect that manifests as starvation intolerance. In order to measure the systemic proteomic response of such deletion in this study, **AlbuVoid™ was chosen as one method to remove albumin from mice and enrich the low abundance proteomes from serum.**

Zheng H, Zhao C, Qian M, Roy S, Arpa A, et al. (2015) AlbuVoid™ Coupled to On-Bead Digestion – Tackling the Challenges of Serum Proteomics. J Proteomics Bioinform 8: 225-230. The AlbuVoid™ bead enrichment is combined with a direct and seamless integration with Trypsin digestion, a method conventionally referred to as on-bead digestion. The digest time was evaluated as a parameter to identify whether different sub-populations of peptides and proteins can be observed by LC-MS analyses. Using 2 different digestion times – 4 hours, and overnight, each with a singular 3 hour gradient LC-MS run, between 400-500 total proteins were observed for both human and rat sera, with overlapping and distinct sub-populations observable at each digest time. **These results support that the described methods gain workflow efficiencies over other high abundance depletion and in-solution digestion workflows**.

Swapan Roy, Matthew Kuruc. The Functional Subproteomes of Serpin Protease Inhibitors are Now Open for LC-MS Biomarker Discovery. MOJ Proteomics Bioinform 2016, 3(6). Using bead-based separation provided by the NuGel™ family of proteomic enrichment products - notably **AlbuVoid™ & AlbuSorb™, the authors demonstrate their utility to satisfy investigations of serum SERPINs.** The authors also suggest their use to develop functional profiles of the SERPIN proteoforms, and how those can establish relationships to disease phenotypes, gene mutations, and dysregulated mechanisms.

David L. Wang, Chuanguang Xiao, Guofeng Fu, Xing Wang and Liang Li. "Identification of potential serum biomarkers for breast cancer using a functional proteomics technology". Biomarker Research (2017) 5:11.
The article states "The **most dramatic difference for enzyme activity detection in using the AlbuVoid™ for serum protein enrichment was demonstrated** in the case of protease activity analysis**.** Compared with the direct serum proteinase measurement, both the levels and species of proteases were increased significantly in the enriched serum sample. …, **and it is necessary to use AlbuVoid™ to enrich these low level proteases to bring them to a high enough level to be detected**."

Cell Culture (BSA Removal)
Jenull, Sabrina, et al. "The histone chaperone HIR maintains chromatin states to control nitrogen assimilation and fungal virulence." *Cell Reports* 36.3 (2021): https://www.sciencedirect.com/science/article/pii/S2211124721008196#mmc1. The authors report a pivotal role for the HIR histone chaperone complex in modulating virulence of the human fungal pathogen *Candida albicans*. The article states for "Cell-free supernatants from 16 hours YNB-BSA (0.025% BSA) cultures grown at 30°C were used for Mass-Spec analysis. Collected supernatants were lyophilized and dissolved in 400 µl of water for **AlbuVoid™** treatment for albumin depletion…**Albumin-free enriched secretory proteome** was eluted from beads".

For a full list of Albumin Removal references, visit:
https://www.biotechsupportgroup.com/References-s/138.htm#albumin-depletion

CONTACT US

We welcome your questions and comments regarding our products.

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Email sales@biotechsupportgroup.com