



BIOTECH SUPPORT GROUP

## AlbuVoid™ LC-MS On-Bead

### For Serum Proteomics

#### *Albumin Depletion Plus Low Abundance Serum Protein Enrichment With Optimized On-Bead Digestion for LC-MS Label and Label-free Analyses*

- Albumin and transferrin voids in flow-through >95%, with <30 minute bind/wash microfuge protocol
- Low abundance enrichment and proteolytic trypsin digestion on the same bead
- Consumable, cost-effective, no column regeneration or cross-contamination
- Species agnostic; human, rat, mouse, goat, sheep, porcine and bovine sera have been tested
- Trypsin digestion on the bead
- Seamless 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

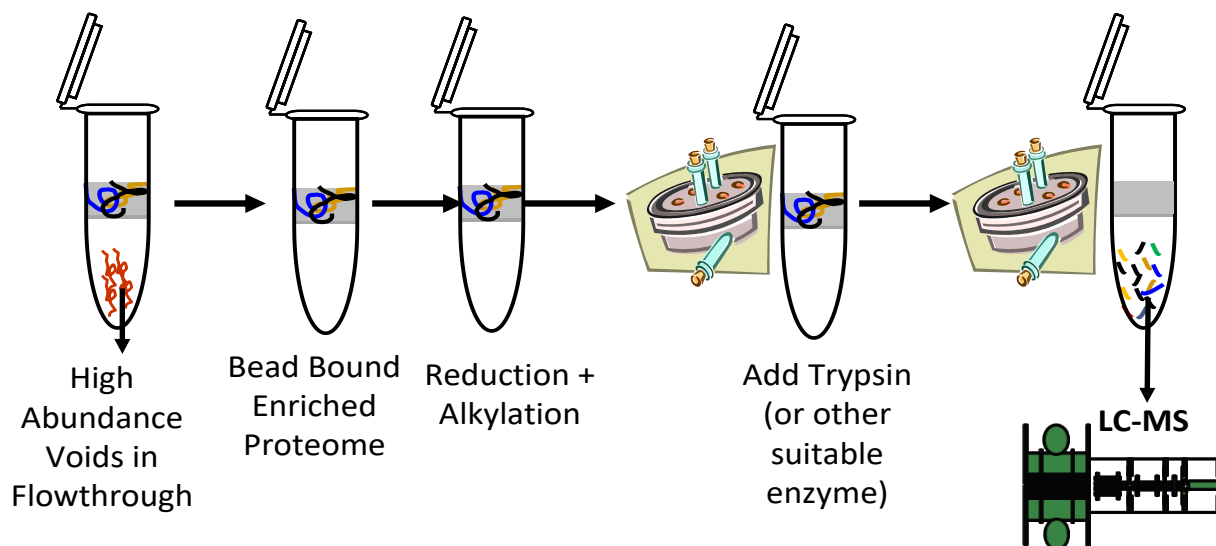
**AlbuVoid™ LC-MS On-Bead** is an albumin depletion kit with protocols especially designed for on-bead proteolytic digestion. Note – the enzyme(s) are not included with the kit. **AlbuVoid™** removes albumin from serum and plasma samples while concentrating low abundance proteins on the beads. As a result, it is ideal for applications involving LC-MS discovery and targeted proteomics.

The **AlbuVoid™** beads are derived from a silica-based library of individual mixed-mode polymeric ligands. The library was designed to facilitate weak binding of proteins, allowing for progressive enrichment of the low abundance proteome, with specialized voiding properties empirically derived. The **AlbuVoid™** beads have been adapted to a protocol specifically designed for LC-MS applications whereby the low abundance proteome adsorbed to the bead is Trypsin degraded to its peptide constituents. In this way **AlbuVoid™ LC-MS On-Bead** integrates low abundance enrichment, with Trypsin (or other suitable protease) on-bead digestion, in a simple, highly efficient and seamless workflow for LC-MS discovery and quantitative analyses.



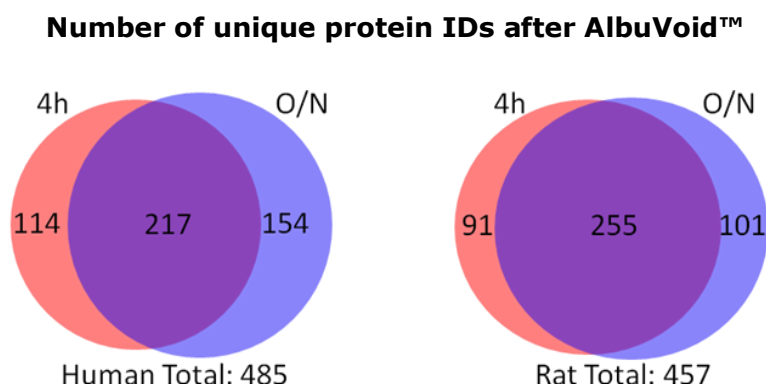
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**High Abundance Depletion + Digestion Efficiency + Simple Workflows = Better LC-MS Output**



### Comparison of 4 hour & Overnight Digestion Times

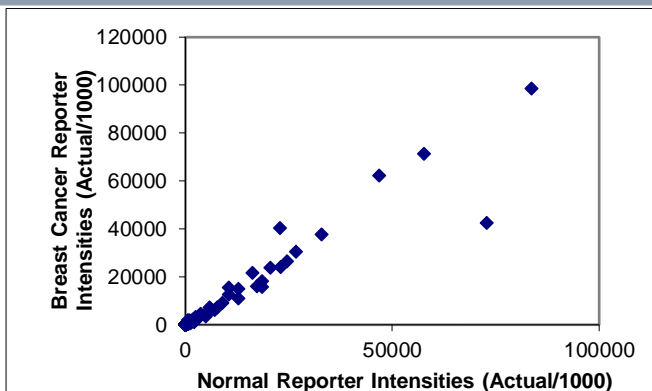
The total **AlbuVoid™ LC-MS On-Bead** proteins were compared for human and rat sera at two different digestion times, 4 hours and overnight (O/N). Note that many identified proteins overlap while certain populations of proteins were only observed in one or the other digest time. The application report is available on-line, entitled: [AlbuVoid™ & On-Bead Digestion: Tackling The Challenges Of Serum Proteomics](#)



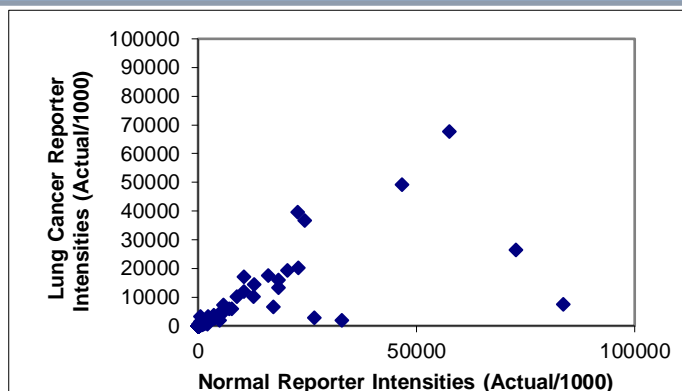


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### A. Breast Cancer Serum Proteins vs. Normal Serum Proteins.

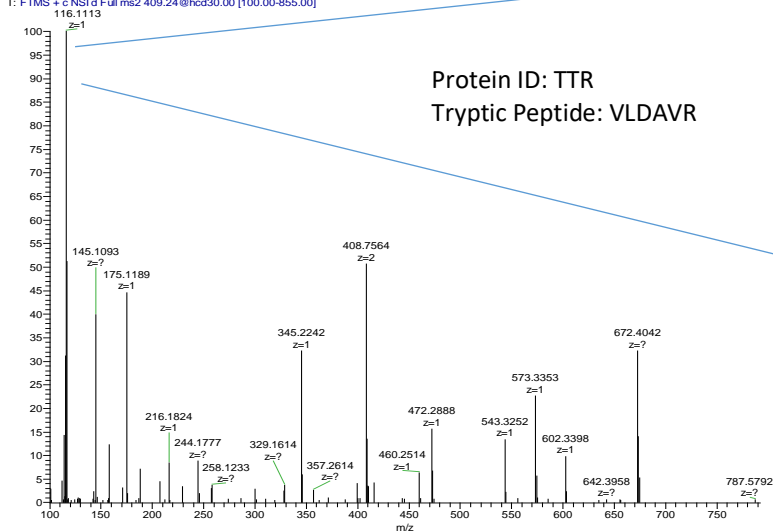


### B. Lung Cancer Serum Proteins vs. Normal Serum Proteins.

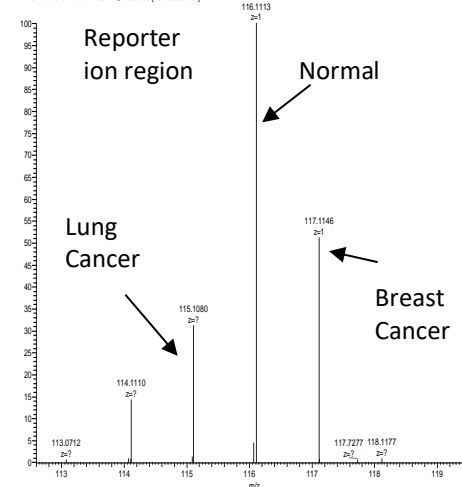


**iTRAQ labeled peptides comparing disease and normal samples.** The reporter intensity signals were added together for each of the iTRAQ labeled peptides supporting the associated protein identification. Each of the additive peptide reporter intensities were then plotted for each protein comparing the 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. As expected, there are only a few outliers in both cases, demonstrating the quantitative efficiency of on-bead digestion.

OE00337 #15329 RT: 53.14 AV: 1 NL: 5.00E5  
T: FTMS + c NSI 4 Full ms2 409.24@hcd30.00 [100.00-855.00]



OE00337 #15329 RT: 53.14 AV: 1 NL: 5.00E5  
T: FTMS + c NSI 4 Full ms2 409.24@hcd30.00 [100.00-855.00]



The **AlbuVoid™ LC-MS On-Bead** product and protocol is compatible with both label and label-free quantification of peptides/proteins. In the example above, iTRAQ labeled peptide from a representative protein, observed to be differentially quantified in comparing pooled sera from normal and cancer patients.



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Product	Size	Total serum/plasma samples processed	Item No.
<b>AlbuVoid™ LC-MS On-Bead</b>	5 Preps	5 x 50-100 µl samples	AVB-MS05
<b>AlbuVoid™ LC-MS On-Bead</b>	10 Preps	10 x 50-100 µl samples	AVB-MS10

Items Required	5 Prep	10 Prep	Reagent
AlbuVoid™ Beads	0.13 gram	0.25 gram	Supplied
Binding Buffer AVBB (0.05M HEPES, pH 6.0)	3 ml	6 ml	Supplied
Wash Buffer AVWB (0.05M HEPES, pH 7.0)	8 ml	15 ml	Supplied
Spin-filter & tube assemblies	5	10	Supplied
DTT, Iodoacetamide, Trypsin and Formic Acid			Not 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 For Albumin Depletion & On bead Digestion For LC-MS Sample Preparation of Serum Proteins

**Processes 50-100 µl serum per prep.** It is recommended that the volume be optimized for the application. For example, for quantitative discovery investigations, smaller volumes may be better, while for total protein annotations or targeted SRM/MRM enrichments, the larger volumes may be optimal.

For best results – the lysate should be clear and free of colloidal material. We recommend first filtering through a 0.45 µm syringe, or microfuge-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 hemoglobin removal.

In bold are the **AlbuVoid™ LC-MS On-Bead** kit components.

- 1. BEAD CONDITIONING.** Weigh out 25 mg of **AlbuVoid™** beads in a spin-filter. Add 150 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.



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2. **SAMPLE PROCESSING.** Add 100 µl of **Binding Buffer AVBB** to beads followed by 100 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).
3. Remove the filtrate as Flow-Through, containing the unbound Albumin.
4. To the beads, add 250 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.

**Option – the proteins can be eluted with (0.25M Tris + 0.5M NaCl, pH 9-10), if other digest protocols or alternative proteomic analysis is preferred. Otherwise, proceed to digest protocol which follows.**

**The bead assisted on-bead digestion protocol (BASP™) is provided below.** The digest buffer is Wash Buffer (0.05M HEPES, pH 7.0). Comparable buffers (0.02-0.5M, pH 6-7) can be used. Higher pH buffers are not recommended.

6. Using **Wash Buffer AVWB**, prepare to 10mM of DTT concentration, and add 100 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 AVWB** twice, to remove any traces of the filtrate.
9. Add 8 µg trypsin in 100 µl **Wash Buffer AVWB** 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 150 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 250µl. 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.



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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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