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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™ 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 the **NRicher™** platform chemistry; a porous silica-bead 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, at or above the sub-proteome saturation of the beads.

The lower abundance enriched sub-proteome that binds to **AlbuVoid™**, can be eluted off without significant carry-over of albumin. It is ideal for applications involving discovery and targeted proteomics, enzyme assays, immunoassay and microarrays, 1D & 2D gel electrophoresis and LC-MS.

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

For targeted proteomics, the **NRicher™** knowledgebase of over 2000 serum proteins is downloadable, and can help select the best product/method(s) for particular protein(s). Go to: https://www.biotechsupportgroup.com/category-s/335.htm

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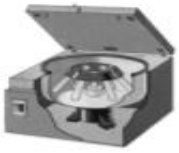
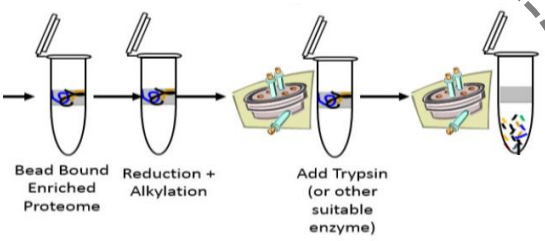
AlbuVoid™ LC-MS On-Bead workflow

High abundance (i.e., Albumin) proteins selectively pass or void through



the beads, concentrating and enriching sub-proteomes on the beads

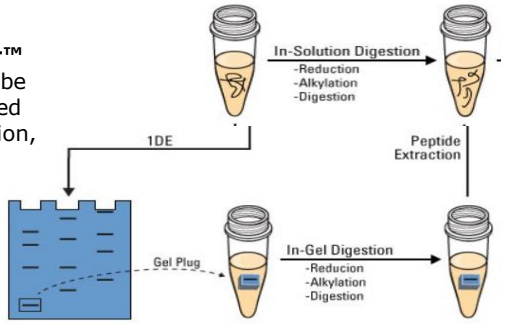
Enriched sub-proteomes remain on the bead and digested using Bead-Assisted Sample Prep (BASP); protocols provided with all **NRicher™** derived products

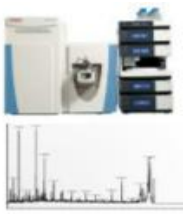


OR

Digest Options

Eluate from all **NRicher™** derived products ™ can be digested by any preferred method, In-Gel, In-Solution, FASP, etc.





LC-MS

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

Items Required	10 Prep	Reagent
AlbuVoid™ Beads	0.25 gram	Supplied
Binding Buffer AVBB (0.05M HEPES, pH 6.0)	6 ml	Supplied
Wash Buffer AVWB (0.05M HEPES, pH 7.0)	15 ml	Supplied
Spin-filter & tube assemblies	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.

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Protocol For Albumin Depletion & On bead Digestion For LC-MS Sample Preparation of Serum Proteins

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. Depending upon the quality of the sample, centrifugation times can be adjusted to increase g's or time, sufficient to process the sample 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.

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, larger volumes may be optimal.

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 1,000 g's. Discard the filtrate. Repeat step-1.
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 g's.
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 g's. 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 4 minutes at 5,000 g's, 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.

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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 4 minutes at 5,000 g's, and retain digested peptides filtrate.

11.To further extract remaining peptides, add 150 µL 10% formic acid, vortex 10 min, centrifuge 4 minutes at 5,000 g's, 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™ References:

Secretome from Cell Culture (BSA Removal)
Paulsen, Bruna, et al. "Reproducible differentiation of pure ovarian support cells from clinical-grade hiPSCs as a novel infertility treatment." *bioRxiv* (2024): 2024-04. To gain insight into the potential mechanism of action of these cells during oocyte maturation beyond transcriptomics readouts, the investigators performed secretome proteomics to investigate proteins that overexpressed in OSC after 24 hours in vitro in comparison with OSCs prior to culture (0 hour) from cells derived, from both CG-hiPSC and RUO-hiPSC. For proteomic analysis, the article states, "The **conditioned media … and then passed through albumin depletion columns (AlbuVoid) to eliminate HSA-derived albumin.**".

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".

Serum/Plasma
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.**

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**.

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.

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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**."

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

For Targeted Proteomics

NRicher™ Bead Platform Provides Unique Sub-Proteome Biases And Fit For Purpose Opportunities for Targeted LC-MS Quantification

Learn more at: https://www.biotechsupportgroup.com/category-s/335.htm

NRicher™ Beads Are Versatile to A Variety of Bead Processing Formats

In addition to standard spin-filter formats, other formats compatible with the 50 µm NRicher™ beads are:

High Throughput Automation Compatible INTip™ SPE (DPX Technologies) Format

Aspirate and dispense cycles mix NRicher™ beads and solutions

The INTip™ SPE tip format improves ease of use and scalability to process multiple samples in parallel, utilizing 96-well plates and automated liquid handlers. The tip-based formats have been proven to be compatible with most automation platforms, i.e., Integra, Hamilton, etc. Please inquire for more information, as these formats are customized to the application and automation platform.

NRicher™ beads can be readily processed in 96-well filter formats. Please inquire.

CONTACT US

We welcome your questions and comments regarding our products.

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