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AlbuVoid™ LC-MS On-Bead For Serum Proteomics

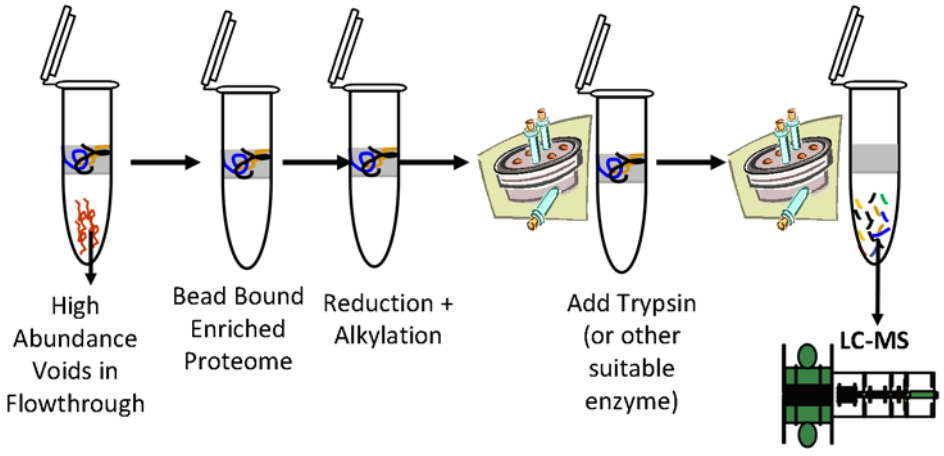
Albumin Depletion Plus Low Abundance Serum Protein Enrichment With Optimized On-Bead Digestion for LC-MS Label and Label-free Analyses

- 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
    - o No in-gel digests, no solution digests, no C18 desalting, more consistent, reproducible results
    - o Compatibility with quantitative label (i.e., iTRAQ) and label-free LC-MS methods

AlbuVoid™ LC-MS On-Bead is an albumin depletion and enzymatic proteolytic digestion reagent kit. It removes albumin from serum and plasma samples while concentrating low abundance proteins on the beads. 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 proteolytically 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.

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



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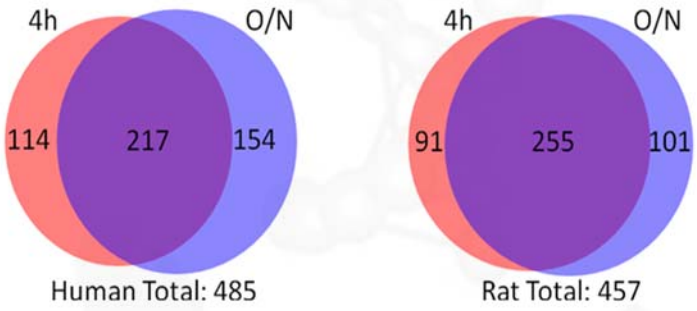
Comparison of 4 hour & Overnight Digestion Times

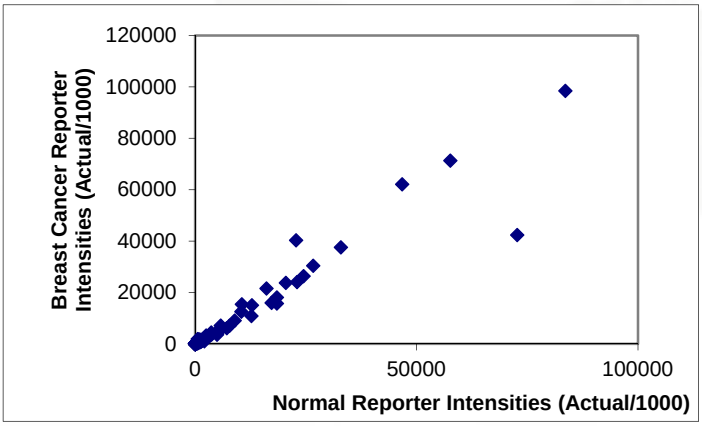
The total **AlbuVoid™ LC-MS On-Bead** proteins were compared for human and rat sera at two different digestion times, 4 hours and overnight (O/N). Note that many identified proteins overlap while certain populations of proteins were only observed in one or the other digest time. The application report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

Number of unique protein IDs after AlbuVoid™



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.

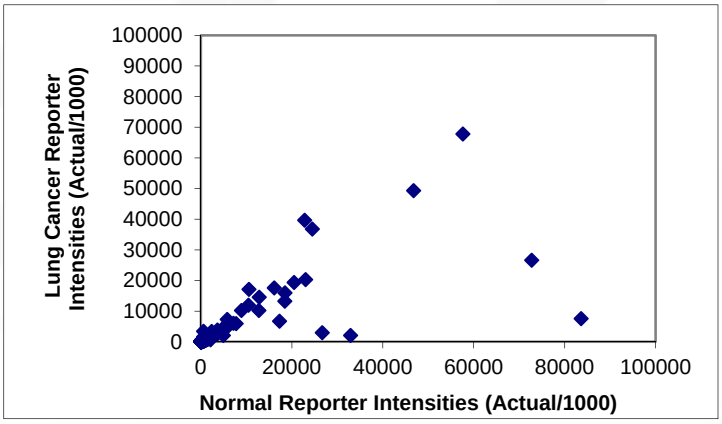


Figure 2 – The reporter intensity signals were added together for each of the iTRAQ labeled peptides supporting the associated protein identification. Each of the additive peptide reporter intensities were then plotted for each protein comparing the following sample pairs: A. Breast Cancer Serum Proteins vs. Normal Serum Proteins. B. Lung Cancer Serum Proteins vs. Normal Serum Proteins. C. Breast Cancer Serum Proteins vs. Lung Cancer Serum Proteins.

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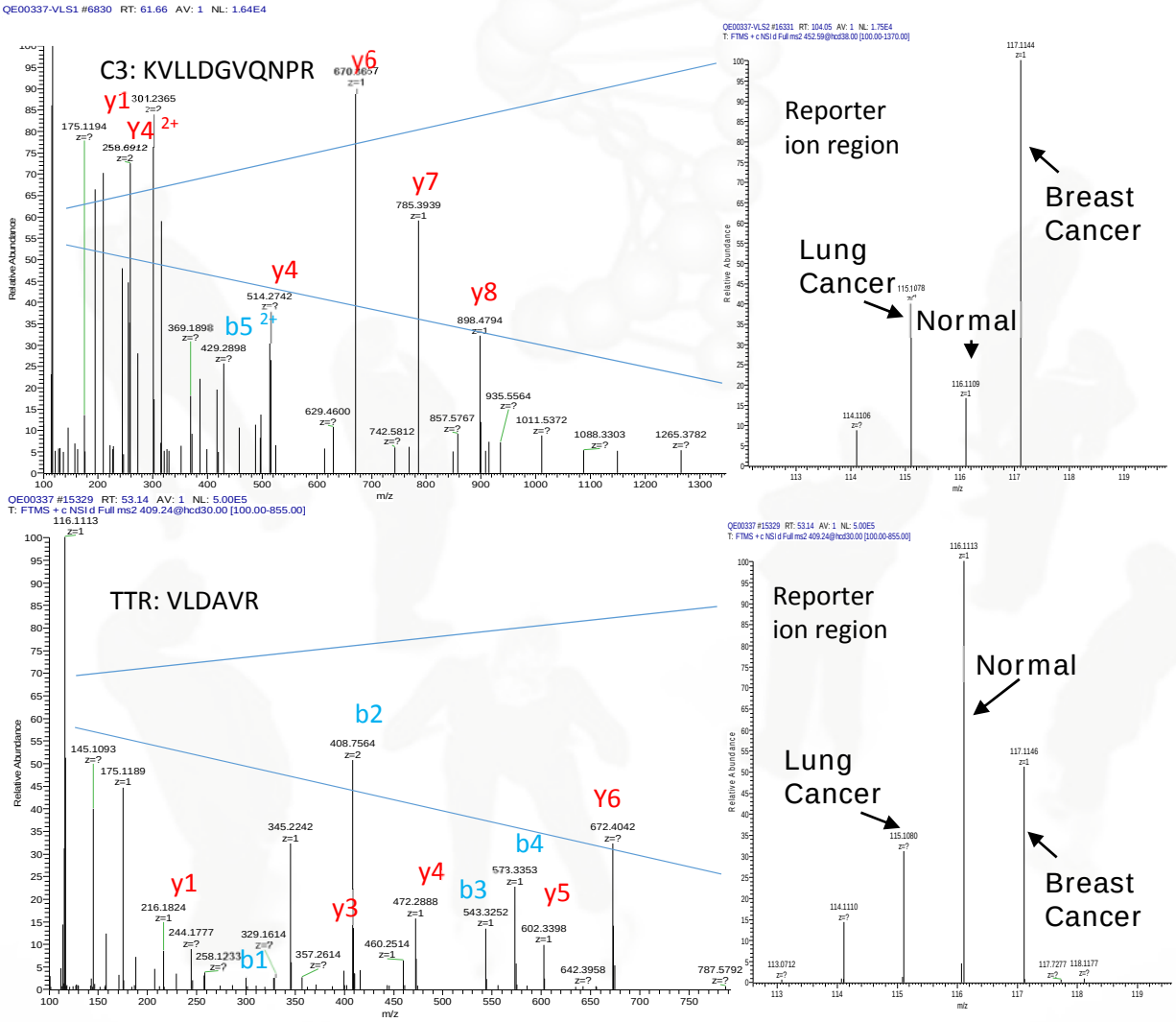
The **AlbuVoid™ LC-MS On-Bead** product and protocol is compatible with both label and label-free quantification of peptides/proteins. In the example below, iTRAQ labeled peptides from two representative proteins observed to be differentially quantified in comparing pooled sera from normal and cancer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



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Product	Size	Total serum / plasma samples processed	Item No.	Price
AlbuVoid™ LC-MS On-Bead	5 Preps	5 x 50-100 µl samples	AVB-MS05	$245
AlbuVoid™ LC-MS On-Bead	10 Preps	10 x 50-100 µl samples	AVB-MS10	$365

Items Supplied	5 Prep	10 Prep	Reagent
AlbuVoid™ Beads	0.25 gram	0.5 gram	Supplied
Binding Buffer AVBB, *PH 6.0*	6 ml	12 ml	Supplied
Wash Buffer AVWB, *PH 7.0*	6 ml	12 ml	Supplied
SpinX Centrifuge tube filters	5	10	Supplied
Trypsin, DTT, Iodoacetamide			Not Supplied

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 serum 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.

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

1. Weigh out 25 mg of **AlbuVoid™** bead in a spin-tube (**0.45µ SpinX centrifuge tube filter supplied**).

2.Add 125 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 100 µl of **AVBB** and 50-100 µl of the Serum. Centrifuge for 5 minutes at 10,000 rpm. Add clarified sample to the **AlbuVoid™ beads** in step 3. Vortex for 10 minutes and then centrifuge for 5 min. at 10,000 rpm.

5. Remove the albumin enriched supernatant (Flow-Through) FT.

6. To the beads, add 250 µl of **Wash Buffer AVWB**. Vortex for 5 min and centrifuge for 4 minutes at 10,000 rpm. Discard the Wash.

7. Repeat Step-6 two times.

The AlbuVoid™ bead is now enriched with albumin depleted low abundance proteins. For LC-MS sample preparation, the on-bead digestion protocol is as follows.

8. After the final wash steps from Step 7 from the enrichment, add 10 µL 100mM DTT + 90 µL **Wash Buffer AVWB**, vortex 10 min, incubate ½ hr at 60 °C.

9.After cooling, add 20µl 200mM Iodoacetamide, and 80 µL **Wash Buffer AVWB**, incubate in dark for 45 min at room temp.

10.Centrifuge at 10,000 rpm (microfuge max setting) for 5 minutes, and discard supernatant.

11.Add 40 µL Sequencing-grade trypsin (0.4µg/µl, in 50mM acetic acid) + 60 µL **Wash Buffer AVWB** to the beads. Digest overnight (maximum) at 37°C or other suitable time period(s).

12. Centrifuge at 10,000 rpm (microfuge max setting) for 5 minutes, and retain peptide filtrate.

13.To further extract remaining peptides, add 150 µL 10% formic acid, vortex 10 min, centrifuge at 10,000 rpm (microfuge max setting) for 5 mins., and add this volume to the first volume.

14.Total is about 250µl. Prepare to desired final concentration. Store at -80 °C until LC-MS/MS.

AlbuVoid™ References:

Serum
Grubbs, J. K., et al. "Investigation of the efficacy of albumin removal procedures on porcine serum proteome profile1." (2015).

Discovery of Functional Serum Biomarkers Using AlbuVoid™ Enrichment and the ArrayBridge PEP Profiling Platform.Personal communication, Xing Wang, Ph.D., ArrayBridge (St. Louis, MO), manuscripts in process.

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Vicki Glaser. Genetic Engineering & Biotechnology News. 2011;31(7):1-55.

Kuruc Matt "Application Report - AlbuVoid™ & On-Bead Digestion - Tackling the challenges of serum proteomics (LC-MS)."

Plasma
Espes, Daniel, Joey Lau, and Per-Ola Carlsson. "Increased circulating levels of betatrophin in individuals with long-standing type 1 diabetes." Diabetologia(2013): 1-4.

Patent - Secretome Sample Preparation
Narain, Niven Rajin, and Paula Patricia Narain. "COMPOSITIONS AND METHODS FOR DIAGNOSIS AND TREATMENT OF PERVASIVE DEVELOPMENTAL DISORDER." U.S. Patent No. 20,150,023,949. 22 Jan. 2015.

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.

Cell Culture
Narain, Niven Rajin, Rangaprasad Sarangarajan, Vivek K. Vishnudas, and Michael Andrew Kiebish. "USE OF MARKERS IN THE IDENTIFICATION OF CARDIOTOXIC AGENTS AND IN THE DIAGNOSIS AND MONITORING OF CARDIOMYOPATHY AND CARDIOVASCULAR DISEASE." U.S. Patent 20,140,100,128, issued April 10, 2014.

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LC-MS Application

New On-Bead Digestion Protocols Improve LC-MS Workflows Of Albumin Depleted Samples
New on-bead digestion for LC-MS applications for proteomic studies. March 11,12, 2013

AlbuVoid™ & On-Bead Digestion Protocols For LC-MS Proteomic Workflows
"Application Report - AlbuVoid™ & On-Bead Digestion - Tackling the challenges of serum proteomics (LC-MS)." April 16, 2015

US HUPO 2014. Frontiers in Proteomics: Advancing Biology through Technology and Computation. AlbuVoid™ abstract entitled "Improved proteomic enrichment and workflow strategies", poster board 089 presented at US HUPO 2014

CONTACT US

We welcome your questions and comments regarding our products.

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