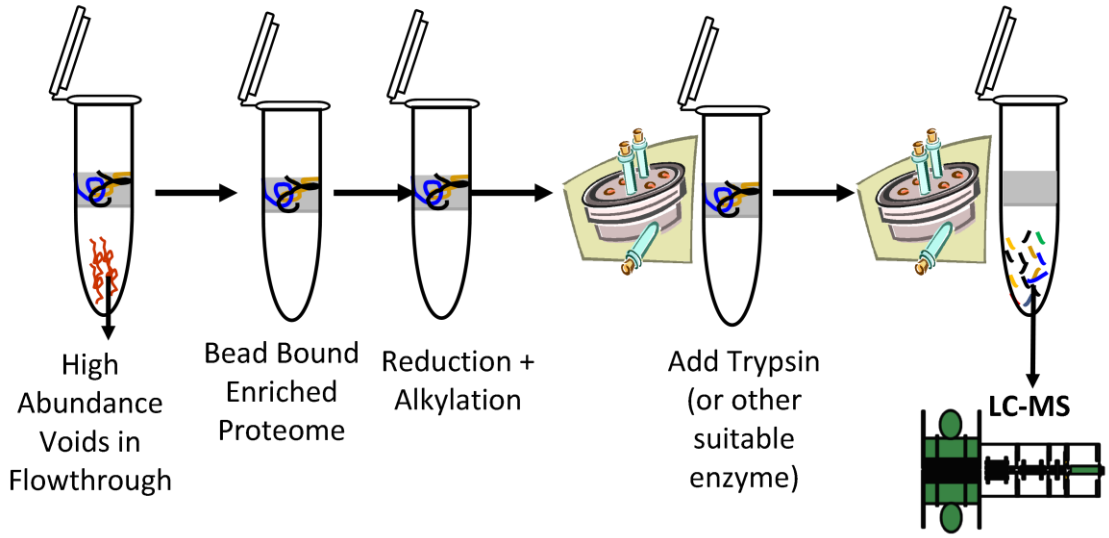
BIOTECH SUPPORT GROUP

AlbuVoid™

Albumin Depleted, On-Bead Digestion Method

Workflow for On-Bead Digestion

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



- Simple, reproducible workflows
- Equivalent or better than in-solution digestion
- Seamless to LC-MS, no desalting or C18 separations

BIOTECH SUPPORT GROUP

PROTOCOL For On Bead Digestion Using AlbuVoid™ Based On Processing 50 - 100 µl Serum

Albumin Depleted On Bead Protein Enrichment

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

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 pellet add 250 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.

On-Bead Digestion Protocol

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 at 37°C or other optimized time period.

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.

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.

BIOTECH SUPPORT GROUP

References:

Serum
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.

Serum Profiling Making Mark on Predictive Medicine
Vicki Glaser.Genetic Engineering & Biotechnology News. 2011;31(7):1-55.

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, Rangaprasad Sarangarajan, and Vivek K. Vishnudas. "INTERROGATORY CELL-BASED ASSAYS AND USES THEREOF." U.S. Patent No. 20,120,258,874. 11 Oct. 2012.

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.

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.

On-Bead Digestion Protocols For LC-MS Proteomic Workflows
New on-bead digestion for LC-MS applications for proteomic studies

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.

Tel: 732-274-2866, 800-935-0628 (North America) Mon – Fri 9am-6pm EST.
Fax 732-274-2899
Email sales@biotechsupportgroup.com
Mail 1 Deer Park Drive, Suite M, Monmouth Junction, NJ 08852
Web: www.biotechsupportgroup.com