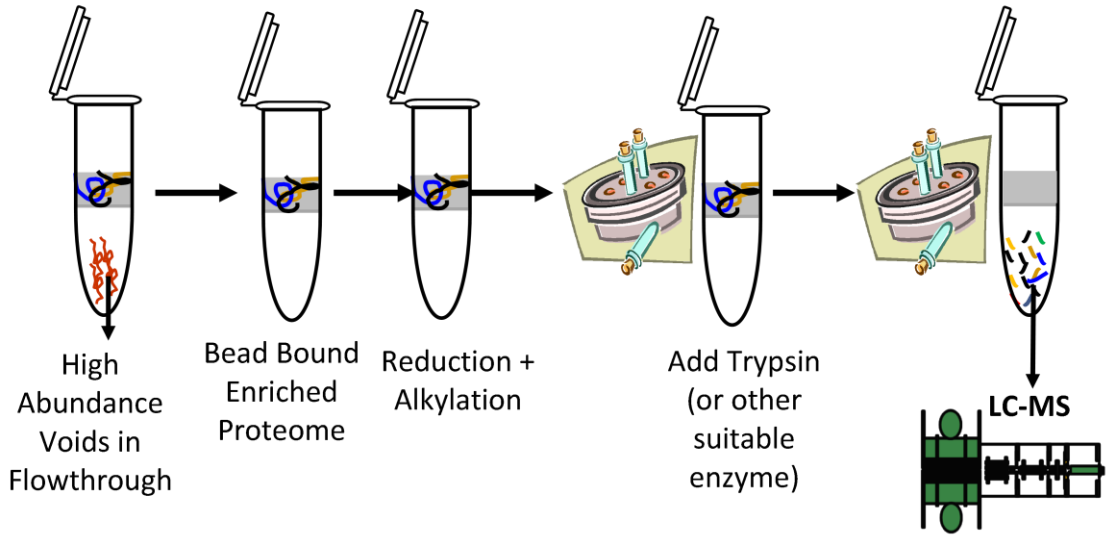
AlbuVoid™

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

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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.

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References:

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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

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