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HemoVoid™ LC-MS On-Bead

For Red Blood Cell and Whole Blood Proteomics

Hemoglobin Depletion Plus Low Abundance Protein Enrichment With Optimized On-Bead Digestion for LC-MS

- Hemoglobin 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

HemoVoid™ LC-MS On-Bead is a hemoglobin depletion kit with protocols especially designed for on-bead proteolytic digestion. Note – the enzyme(s) are not included with the kit. HemoVoid™ removes hemoglobin from red blood cell or whole blood lysates while concentrating low abundance proteins on the beads. It is ideal for applications involving LC-MS discovery and targeted proteomics.

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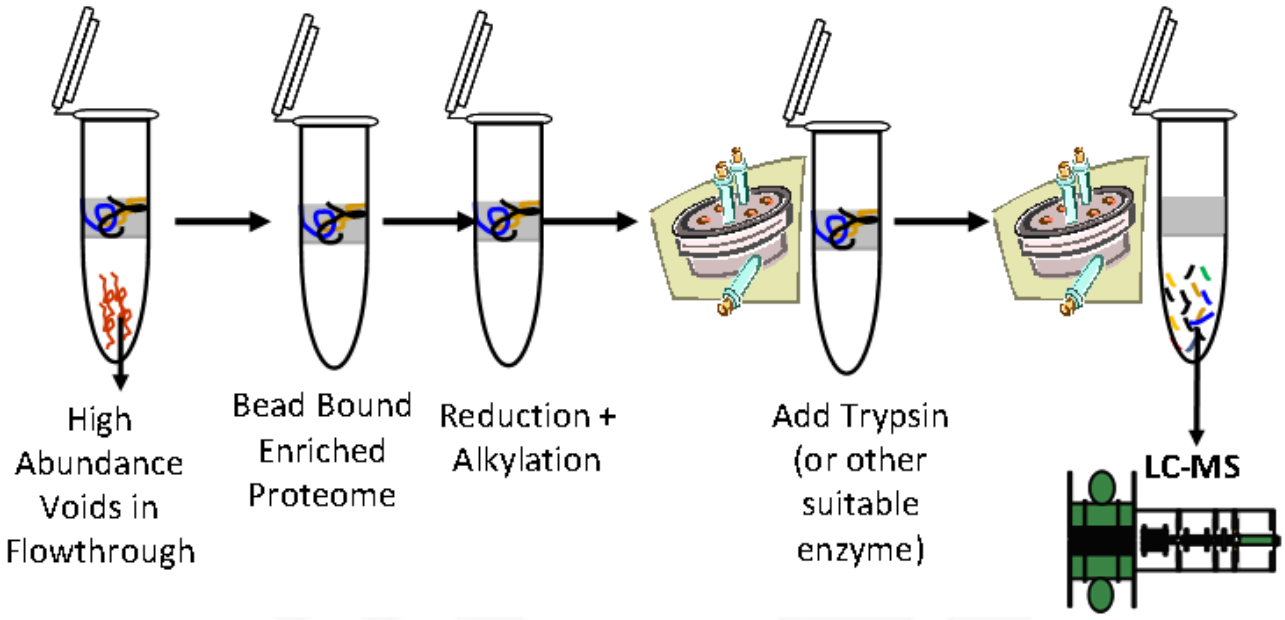
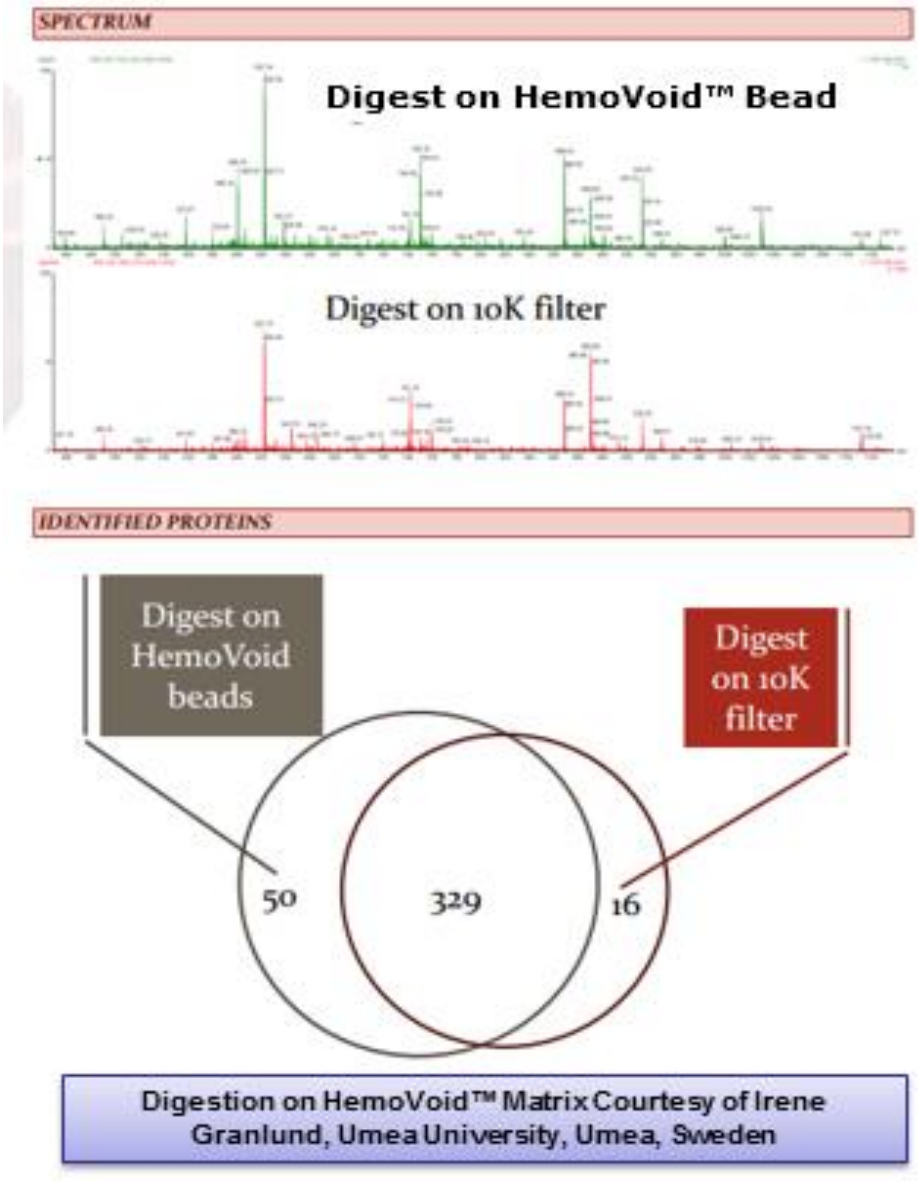
High Abundance Depletion + Digestion Efficiency + Simple Workflows = Better LC-MS Output




SPECTRUM
Digest on HemoVoid™ Bead
Digest on 10K filter
IDENTIFIED PROTEINS
Digest on HemoVoid beads
Digest on 10K filter
50
329
16
Digestion on HemoVoid™ Matrix Courtesy of Irene Granlund, Umea University, Umea, Sweden

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Product	Size	Total RBC samples processed	Item No.
HemoVoid™ LC-MS On-Bead	5 Preps	5 x 100-200 µl samples	HVB-MS05
HemoVoid™ LC-MS On-Bead	10 Preps	10 x 100-200 µl samples	HVB-MS10
NOTE: Please contact sales@biotechsupportgroup.com for prices in bulk quantities.			

Items Required	5 Prep	10 Prep	Reagent
HemoVoid™	0.13 gram	0.25 gram	**Supplied**
Binding Buffer HVBB, *PH 6.0*	5 ml	10 ml	**Supplied**
Wash Buffer HVWB, *PH 7.0*	10 ml	20 ml	**Supplied**
SpinX Centrifuge tube filters	5	10	**Supplied**
Trypsin, DTT, Iodoacetamide			**Not Supplied**

PROTOCOL For On Bead Digestion Using HemoVoid™ Based On Processing 100-200 µl Red Blood Cell (RBC) Lysate

Processes 100-200 µl red blood cell lysate 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-type filter before beginning the prep.

1. Weigh out 25 mg of **HemoVoid™** matrix in a spin-tube.

2. Add 150 µl of **Binding Buffer HVBB**. Vortex or mix well for 5 minutes at room temperature followed by centrifugation for 2 minutes at 3000 rpm. Discard the supernatant.

3. Repeat step-2

4. Add 150 µl of **HVBB** and 100-200 µl of the **Sample.** Vortex for 10 min and then centrifuge for 4 minutes at 10,000 rpm.

5. Remove the filtrate as Flow-Through **FT**.

6. To the pellet, add 250 µl of **Wash Buffer HVWB.** Vortex or mix well for 5 min and centrifuge for 4 minutes at 10000 rpm. Remove the filtrate as **Wash.**

7. Repeat Step-6, 2 times.

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 HVWB**, vortex 10 min, incubate ½ hr at 60 °C.

9.After cooling, add 20µl 200mM Iodoacetamide, and 80 µL **Wash Buffer HVWB**, 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 HVWB** 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.

References

A complete Albumin Removal Reference Report can be downloaded at:
http://biotechsupportgroup.com/sites/default/files/Hemoglobin%20Removal%20App%20Alert.pdf

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Sudha Neelam, David G Kakhniashvili, Stephan Wilkens et al. Functional 20S proteasomes in mature human red blood cells Experimental Biology and Medicine.2011;236:580-591

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