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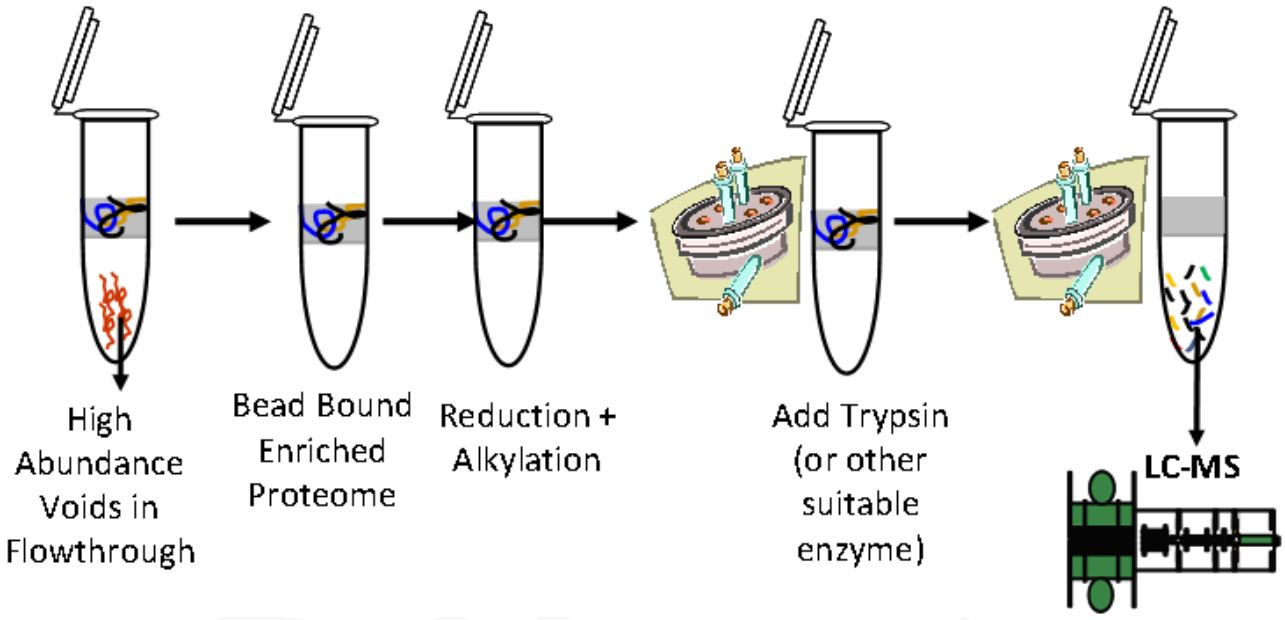
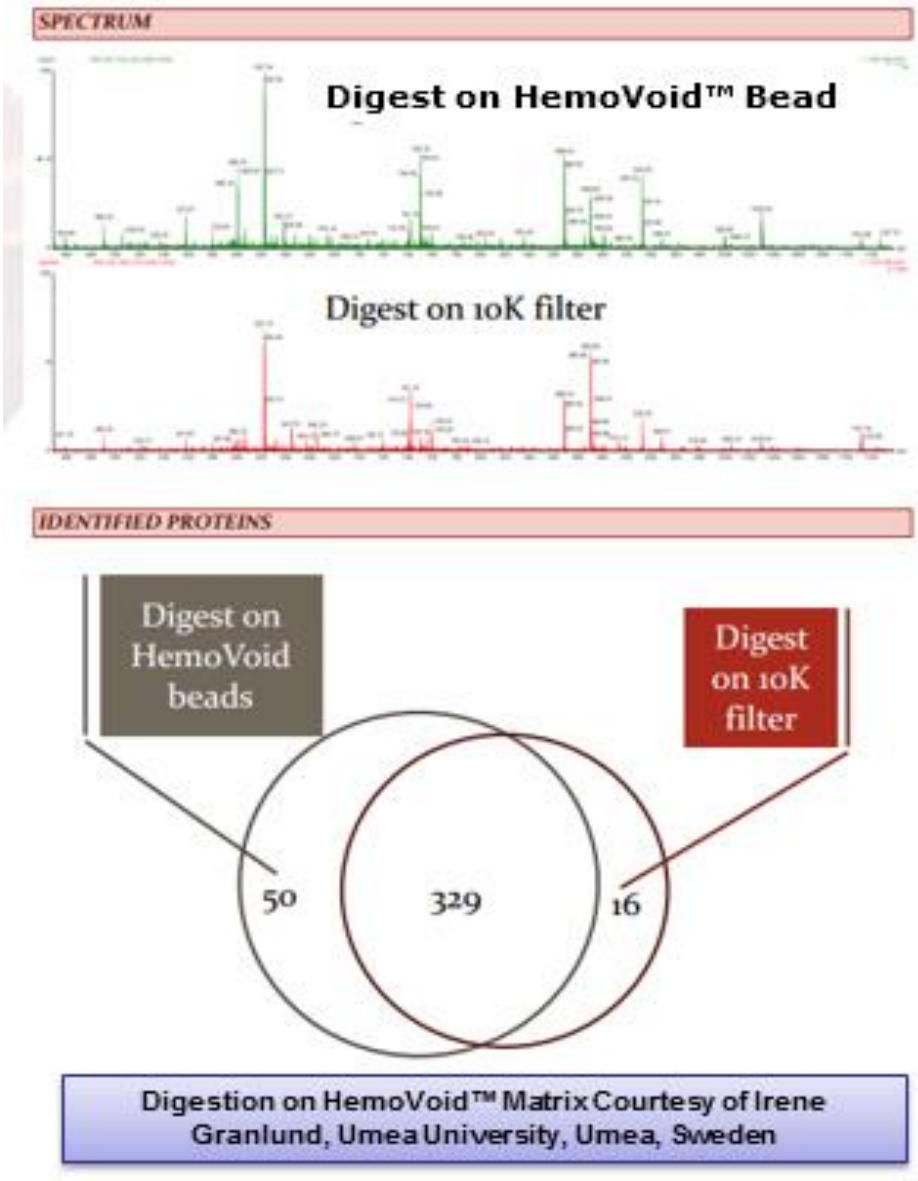
High Abundance Depletion + Digestion Efficiency + Simple Workflows = Better LC-MS Output




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

Items Required	5 Prep	10 Prep	Reagent
HemoVoid™	0.13 gram	0.25 gram	**Supplied**
Binding Buffer HVBB, *PH 6.0*	3 ml	5 ml	**Supplied**
Wash Buffer HVWB, *PH 7.0*	5 ml	10 ml	**Supplied**
SpinX Centrifuge tube filters	5	10	**Supplied**
Trypsin, DTT, Iodoacetamide			**Not Supplied**

PROTOCOL

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.

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On-Bead Digestion Protocol

The HemoVoid™ bead is now enriched with albumin depleted low abundance proteins. For LC-MS sample preparation, the on-bead digestion protocol is as follows. Option – the proteins can be eluted with HVEB, provided upon request.

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.

Featured HemoVoid™ Reference Applications

Human Red Blood Cells (RBC)

Bollenbach, Alexander, et al. "GC-MS and LC-MS/MS pilot studies on the guanidine (NG)-dimethylation in native, asymmetrically and symmetrically NG-dimethylated arginine-vasopressin peptides and proteins in human red blood cells." *Journal of Chromatography B* (2020): 122024.

Previous studies showed that human red blood cells are rich in large (> 50 kDa) asymmetric dimethylarginine -containing proteins of unknown identity. The study aimed to report the identity, biological activity and concentration of *N*G-methylated proteins by using GC-MS and LCMS/MS approaches. The article states "we included in our method the use of HemoVoid™ to remove specifically most erythrocytic hemoglobin and to improve the SDS-PAGE separation of proteins for further processing. The HemoVoid™, … allowed removal of erythrocytic hemoglobin to a large extent from the hemolysate. … removal of hemoglobin by this technique enabled an effective separation by SDS-PAGE and isolation of bands, presumably by avoiding overloading of the gels by hemoglobin.".

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Kitao, Akihito, et al. "Band 3 ectopic expression in colorectal cancer induces an increase in erythrocyte membrane-bound IgG and may cause immune-related anemia." *International Journal of Hematology* (2020): 1-10.

Autoimmune hemolytic anemia (AIHA) is a rare comorbidity in colorectal cancer (CRC) and has an unknown etiology. To better understand cancer-related anemia, the authors' investigated ectopic band 3 expression and erythrocyte membrane-bound IgG in a CRC cohort. To reduce the interference from Hemoglobin, the article states "Erythrocytes were lysed … and hemoglobin was depleted using HemoVoid (Biotech Support Group, NJ, USA, Cat. No. HVK-10)".

Rosin-Arbesfeld, Rina, and Ronen SIMAN-TOV. "Article of manufacture and methods for increasing survival of red blood cells." U.S. Patent Application No. 15/739,857.

The patent application describes an ex - vivo method of increasing survival of red blood cells (RBCs). The method comprises contacting the RBCs with an activator of the non - canonical Wnt pathway, which results in actin polymerization, thereby increasing survival of RBCs. The invention's description states "The Haemolysates were enriched with over 95 % hemoglobin. For hemoglobin depletion, the hemoglobin depletion kit of HemoVoid … was used". Upon depletion of hemoglobin, a reduction in cytoplasmic actin levels was observed.

Nemkov, Travis, et al. "Hypoxia modulates the purine salvage pathway and decreases red blood cell and supernatant levels of hypoxanthine during refrigerated storage." *haematologica* 103.2 (2018): 361-372.

The goal of this study was to use proteomics in part to understand hypoxanthine catabolism *in vivo* for stored red blood cells. It is still unclear whether accumulation of hypoxanthine in stored red blood cell units is clinically relevant for transfused recipients. The article states "Leukocyte-reduced human RBC from healthy donor volunteers were washed five times in phosphate-buffered saline prior to lysis in distilled water with sonication. Proteomic analyses of RBC membranes and cytosols were performed…RBC cytosolic proteins were depleted of hemoglobin using Hemovoid™ (Biotech Support Group, Monmouth Junction, NJ, USA), prior to high-pH reversed phase fractionation".

Cortese-Krott, Miriam M., et al. "Identification of a soluble guanylate cyclase in RBCs: preserved activity in patients with coronary artery disease." *Redox Biology* (2017). http://www.sciencedirect.com/science/article/pii/S2213231717306535

In brief, the authors aimed to investigate whether RBCs carry a functional soluble guanylate cyclase (sGC) signalling pathway and to address whether this pathway is compromised in coronary artery disease. The article states "Using a commercial resin (HemoVoid™), which removes hemoglobin… and allows enrichment of soluble cytoplasmic proteins, we established a procedure that allows fast and reliable preparation of hemoglobin-free cell lysates from as little as 1-2 ml blood. In those samples, expression and activity of the cGMP-generating sGC, cGMP-hydrolyzing PDE5 and cGMP-transducing PKG was assessed by enzymatic assays and Western blot analysis".

Feliciano, Amélia, et al. "Evening and morning alterations in Obstructive Sleep Apnea red blood cell proteome." *Data in Brief* (2017). http://dx.doi.org/10.1016/j.dib.2017.01.005

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Using proteomics-based evaluation of red blood cells (RBC), the authors identified differentially abundant proteins associated with Obstructive Sleep Apnea Syndrome (OSA). Proteome variations between various time points were assessed. The article states "RBC cytoplasmic fraction depleted of hemoglobin, using HemoVoid™ system, were analyzed by two-dimensional fluorescence difference gel electrophoresis (2D-DIGE), the 2D image software-based analyzed and relevant differentially abundant proteins identified by mass spectrometry (MS)".

Philipp F Lange, Pitter F Huesgen, Karen Nguyen, and Christopher M Overall. " *Annotating N termini for the Human Proteome Project: N termini and Nα-acetylation status differentiate stable cleaved protein species from degradation remnants in the human erythrocyte proteome",* J. Proteome Research., Just Accepted Manuscript • DOI: 10.1021/pr401191w • 21 Feb 2014

The article describes a goal of the Chromosome-centric Human Proteome Project to identify all human protein species. Enucleated, erythrocytes are simple yet proteomically challenging cells due to the high hemoglobin content (about 97% by mass) and wide dynamic range of protein concentrations that impedes protein identification. Using a N-terminomics procedure called TAILS, the authors identified from the HemoVoid™ treated, soluble fraction, 778 proteins were identified, 171 of which were not represented in either the soluble non-depleted fraction or the membrane fraction.

Barasa, Benjamin, and Monique Slijper. "Challenges for red blood cell biomarker discovery through proteomics." *Biochimica et Biophysica Acta (BBA)-Proteins and Proteomics* 1844.5 (2014): 1003-1010.

Katja Walpurgis, Maxie Kohler, Andreas Thomas et al. Validated hemoglobin-depletion approach for red blood cell lysate proteome analysis by means of 2D-PAGE and Orbitrap MS.Electrophoresis.2012;

Mizukawa, B., George, A., Pushkaran, S. et al. Cooperating G6PD mutations associated with severe neonatal hyperbilirubinemia and cholestasis.Pediatric Blood Cancer.2011;56: 840-842.

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

HemoVoid™ On Bead Digestion Application Work On RBC by Irene Granlund, *Umeå University*

RBCs in Parkinson's Disease

Klatt, Stephan, et al. "Optimizing red blood cell protein extraction for biomarker quantitation with mass spectrometry." *Analytical and Bioanalytical Chemistry* (2020): 1-14.

The article describes the advantage of HemoVoid™ in detection of low abundance proteins when comparing their amounts (in percent) between four alternative extraction conditions, stating "… Most peptides, following HemoVoid™ extraction, showed ion abundances ranging between 1.00E+5 and 1.00E+6 (31%). In comparison to this, fewer peptides (10–23%) were within this range following extraction with all other protocols". With respect to potential biomarkers for Parkinson's Disease, the article states "For example, PRDX6 accounts for 0.4% of the total ion abundance after DOC

(deoxycholate) extraction, whereas following HV (HemoVoid™) extraction, this increases to 8%, a 20-fold enrichment". The authors conclude that the HemoVoid™ method significantly reduces the concentration of hemoglobin, resulting in an increased signal-to noise of the remaining red cell proteins. The article describes methods to digest the HemoVoid™ bead-bound proteome, greatly simplifying the workflow for LC-MS/MS analysis.

Elhadi, Suaad Abd, et al. "α-Synuclein in blood cells differentiates Parkinson's disease from healthy controls." *Annals of Clinical and Translational Neurology*.

The goal of this study was to determine whether blood cells expressing α-Synuclein can differentiate Parkinson's disease (PD) from healthy controls. Two proteoforms - PSer129 a-Syn (phosphorylated pathological form in Lewy bodies) and Oxidized a-Syn levels are observed in blood cells, but both at considerably lower concentration than total a-Syn, so the extremely high abundance of hemoglobin interferes with their analysis. To compensate, the article states for PSer129 α -Syn & Oxidized α -Syn detection by immunoassay, "followed from hemoglobin clearance with HemoVoid kit (Biotech Support Group LLC, NJ, US)".

Red Blood Cells, Plasmodium extracts

Machado, Patrícia Isabel Pires. *Pyruvate kinase and glucose-6-phosphate dehydrogenase deficiencies and their association with malaria–population genetics and proteomic studies*. Diss. Universidade do Porto, 2013.

Walpurgis, Katja, et al. "Effects of gamma irradiation and 15 days of subsequent ex vivo storage on the cytosolic red blood cell proteome analyzed by 2D DIGE and Orbitrap MS." *PROTEOMICS-Clinical Applications* (2013).

P. Falciparum Clone 3D7 Cultured In Human Erythrocytes

Lasonder E, Green JL, Camarda G, Talabani H, Holder AA, Langsley G, Alano P. The Plasmodium falciparum schizont phospho-proteome reveals extensive phosphatidylinositol and cAMP-Protein Kinase A signalling. J Proteome Research. 2012;

Species Agnostic – Applications in non-human samples

Puente-Marin, Sara, et al. "In Silico Functional Networks Identified in Fish Nucleated Red Blood Cells by Means of Transcriptomic and Proteomic Profiling." *Genes* 9.4 (2018): 202.

Nucleated red blood cells (RBCs) of fish have, in the last decade, been implicated in several immune-related functions, such as antiviral response, phagocytosis or cytokine-mediated signaling. Label-free shotgun proteomic analyses were carried out for in silico functional pathway profiling of rainbow trout RBCs. The article states "The cytosolic fraction, approximately 300 µL, was depleted of hemoglobin using HemoVoid™ kit (Biotech Support Group, Monmouth Junction, NJ, USA), in accordance with the manufacturer's instructions".

Nombela I, Puente-Marin S, Chico V et al. Identification of diverse defense mechanisms in trout red blood cells in response to VHSV halted viral replication [version 1; referees: awaiting peer review]. F1000Research 2017, 6:1958 (doi: 10.12688/f1000research.12985.1)

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Fish nucleated red blood cells (RBCs) generate a wide variety of immune-related gene transcripts when viruses replicate inside them and are their main target cell. However, the objective of this study not yet explored, was to determine the immune response and mechanisms of fish RBCs against viruses targeting other cells or tissues. The article states "a new proteomic analysis method was carried out that combines fractionation into cytosolic and membrane fractions, haemoglobin removal of the cytosolic fraction, protein digestion, pH reversed-phase peptide fractionation and finally LC ESI-MS/MS analysis of each of the fractions… . Briefly, the haemoglobin of the cytosolic fraction was removed using a column of HemoVoid™ kit (Biotech Support Group, Monmouth Junction, NJ), following the manufacturer instructions".

CONTACT US

We welcome your questions and comments regarding our products.

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