BIOTECH SUPPORT GROUP

HemoVoid™ LC-MS On-Bead

For Red Blood Cell and Whole Blood Proteomics

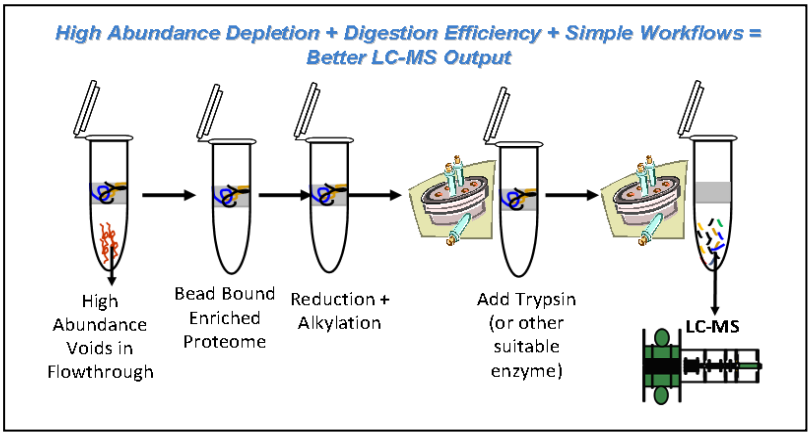
Hemoglobin Depletion Plus Low Abundance Protein Enrichment With Optimized On-Bead Digestion for LC-MS

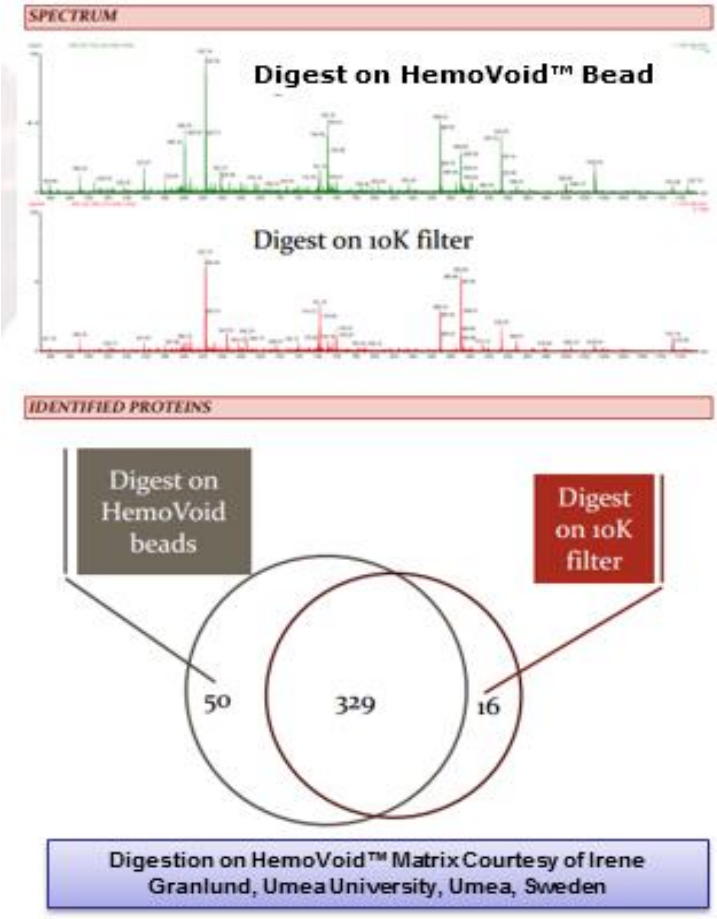
- Hemoglobin voids in flow-through >98%, with <30 minute bind/wash/elute protocol
- Hemoglobin removal from red cell lysates for RBC proteomics
- Hemoglobin removal from whole blood lysates and hemolyzed serum
- Disposable, cost-effective
- Species agnostic, validated on human, sheep, bovine, goat, fish, etc.
- For LC-MS, optional seamless On-bead protocols (BASP™) 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 imperfect fit polymeric ligands. The library was designed to facilitate weak binding of proteins, allowing for preferential displacement of the stronger bias binding proteins, 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 Trypsin 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.

BIOTECH SUPPORT GROUP





BIOTECH SUPPORT GROUP

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™ Beads	0.13 gram	0.25 gram	Supplied
Binding Buffer HVBB (0.05M HEPES, pH 6.0)	3 ml	6 ml	Supplied
Wash Buffer HVWB (0.05M HEPES, pH 7.0)	8 ml	15 ml	Supplied
Spin-filter & tube assemblies	5	10	Supplied
DTT, Iodoacetamide, Trypsin and Formic Acid			Not Supplied

Additional Spin-Filters (low protein binding, 0.45 µm filter element) can be purchased separately, please inquire.

If there are any questions about compatibility or substitution with other buffers, please contact us.

PROTOCOL

Based On Processing 100-200 µl Red Blood Cell (RBC) Lysate

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, or microfuge-type filter before beginning the prep.

The centrifugation time may vary, adjust as necessary to get complete filtration through the beads.

The protocol can be scaled up or down proportionally to adjust for different volumes. The bead amount can be adjusted to accommodate more or less hemoglobin removal.

1. **BEAD CONDITIONING.** Weigh out 25 mg of **HemoVoid™** matrix in a spin-tube. Add 150 µl of **Binding Buffer HVBB**. Vortex or mix thoroughly for 5 minutes at room temperature followed by centrifugation for 2 minutes at 5,000-10,000 rpm (2,000-8,000xg). Discard the filtrate. Repeat step-1.

BIOTECH SUPPORT GROUP

2. **Sample Processing.** Add 150 µl of **Binding Buffer HVBB** to beads followed by 100-200 µl of the **Sample.** Vortex or mix thoroughly for 10 min and then centrifuge for 4 minutes at 5,000-10,000 rpm (2,000-8,000xg).

3. Remove the filtrate as Flow-Through, containing the unbound Hemoglobin.

4. To the beads, add, add 250 µl of **Wash Buffer HVWB.** Vortex or mix well for 5 min and centrifuge for 4 minutes at 5,000-10,000 rpm (2,000-8,000xg). Discard the filtrate as **Wash.**

5. Repeat Step-4, 2 times. The **HemoVoid™** beads contain the enriched Hemoglobin-depleted sub-proteome.

Option – the proteins can be eluted with (0.25M Tris + 0.5M NaCl, pH 9-10), if other digest protocols or alternative proteomic analysis is preferred. Otherwise, proceed to digest protocol which follows.

The bead assisted on-bead digestion protocol (BASP™) is provided below. The digest buffer is Wash Buffer (0.05M HEPES, pH 7.0). Comparable buffers (0.02-0.5M, pH 6-7) can be used. Higher pH buffers are not recommended.

6. Using **Wash Buffer HVWB**, prepare to 10mM of DTT concentration, and add 100 µl to the beads and vortex for 10 minutes and incubate for 30 minutes at 60C.

7. Cool the samples to RT, add suitable volume of Iodoacetamide to 20mM and incubate in the dark for 45 minutes

8. Centrifuge at 5,000-10,000 rpm (2,000-8,000xg) for 5 minutes, and discard filtrate. Rinse the bottoms of the spin-filter tubes with 500 µl of 50% ACN, **Wash Buffer HVWB** twice, to remove any traces of the filtrate.

9. Add 8 µg trypsin in 100 µl **Wash Buffer HVWB** to the beads and keep at 37°C for a minimum 4 hours to maximum overnight. Overnight is recommended to start with. In select targeted circumstances, 2 hours may be sufficient.

10. Centrifuge at 5,000-10,000 rpm (2,000-8,000xg) for 5 minutes, and retain digested peptides filtrate.

11.To further extract remaining peptides, add 150 µL 10% formic acid, vortex 10 min, centrifuge at 5,000-10,000 rpm (2,000-8,000xg) for 5 mins., and combine this volume with volume from step 10.

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

BIOTECH SUPPORT GROUP

Selection of HemoVoid ™ Reference Applications

Red Blood Cells (RBC) Lysates

Liu, Wenli, et al. **"**Erythroid lineage Jak2 V617F expression promotes atherosclerosis through erythrophagocytosis and macrophage ferroptosis**."** *The Journal of Clinical Investigation* (2022).

To investigate whether selective erythroid Jak2VF expression promotes atherosclerosis, the study used hyperlipidemic Erythropoietin Receptor Cre mice that express Jak2VF in the erythroid lineage (VFEpoR mice). To explore underlying defects promoting oxidative changes in Jak2VF Red Blood Cells (RBC), unbiased proteomics profiling was conducted. The article states "…**haemoglobin removal and on-bead digestion, …was based on the protocol in HemoVoid kit**…" The proteomic data showed a prominent increase in EIF2AK1 (also known as heme-regulated inhibitor, HRI) and EIF2A (Left), consistent with increased oxidative stress.

Jing, Lun, et al. "PROTEOMIC ANALYSIS IDENTIFIED LBP AND CD14 AS KEY PROTEINS IN BLOOD/BIPHASIC CALCIUM PHOSPHATE MICROPARTICLE INTERACTIONS." *Acta Biomaterialia* (2021).

In a LC-MS/MS proteomic study, the authors compared the differentially expressed blood proteins (plasma and blood cell proteins) and the deregulated signaling pathways of osteogenic and fibrogenic blood composites. The article describes use of HemoVoid™ for depletion of Hemoglobin prior to LC-MS analysis, "each composite material or 4 blood clots were pooled into 3ml of cooled lysis buffer [HEPES 50mM (pH 7.4); NaCl 150mM; EDTA 20mM (pH 8); CHAPS 1%; DTT 1mM; Protease and Phosphatase inhibitor cocktail]. …The supernatants were collected, and hemoglobin depleted using several HemoVoid™ columns (Biotech Support Group). When indicated, albumin was also partially removed using AlbuVoid™ depletion reagent kit (Biotech Support Group) following the manufacturer's instructions." **From these enrichment steps, the investigators found respectively 80 and 92 proteins differentially expressed between blood clot and BCP 80-200 or BCP 200-500 blood composites.**

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.
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….".

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 "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), …".

BIOTECH SUPPORT GROUP

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

Using proteomics-based evaluation of red blood cells (RBC), the authors identified differentially abundant proteins associated with Obstructive Sleep Apnea Syndrome (OSA. 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**.

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)".

BIOTECH SUPPORT GROUP

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 F1000Research 2017, 6:1958 (doi: 10.12688/f1000research.12985.1)
The objective of this study 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".

For a full list of Hemoglobin Removal References, visit:
https://www.biotechsupportgroup.com/References-s/138.htm#hemoglobin-depletion

RELATED SAMPLE PREP PRODUCTS:

Albumin & IgG Removal products:
https://www.biotechsupportgroup.com/Albumin-Removal-s/307.htm

Lipid Removal Reagent and Clarification products:
https://www.biotechsupportgroup.com/Lipid-Removal-s/316.htm

CONTACT US

We welcome your questions and comments regarding our products.

Call 732-274-2866, 800-935-0628 (North America) Mon – Fri 9am-6pm EST.
Email sales@biotechsupportgroup.com