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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 >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™ is a Hemoglobin depletion reagent kit, however the beads do not bind Hemoglobin. It removes Hemoglobin from whole blood or red cell lysates while enriching low abundance proteins on the beads. The **HemoVoid™** protocol uses mild buffers; the protocol conditions are very gentle so that native enzyme and functional activity is retained in elution fractions.

HemoVoid™ beads are derived from the **NRicher™** platform chemistry; a porous silica-bead 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, at or above the sub-proteome saturation of the beads.

The lower abundance enriched sub-proteome that binds to **HemoVoid™**, can be eluted off without significant carry-over of Hemoglobin. It is ideal for applications involving discovery and targeted proteomics, enzyme assays, immunoassay and microarrays, 1D & 2D gel electrophoresis and LC-MS.

In addition, all **NRicher™ beads, including HemoVoid™** have been adapted to a protocol specifically designed for LC-MS applications whereby the low abundance proteome adsorbed to the beads can be Trypsin processed to its peptide constituents. This is called Bead Assisted Sample Prep or BASP™; the protocol is included as an optional digest method.

For targeted proteomics, the **NRicher™** knowledgebase of over 2000 serum proteins is downloadable, and can help select the best product/method(s) for particular protein(s). Go to: https://www.biotechsupportgroup.com/category-s/335.htm

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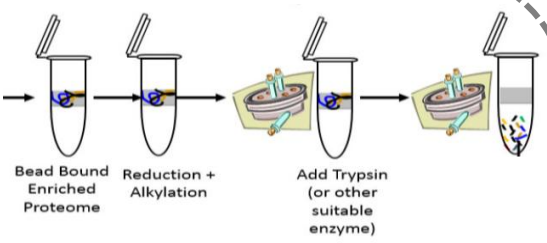
HemoVoid™ LC-MS On-Bead workflow

High abundance (i.e., Albumin) proteins selectively pass or void through

the beads, concentrating and enriching sub-proteomes on the beads

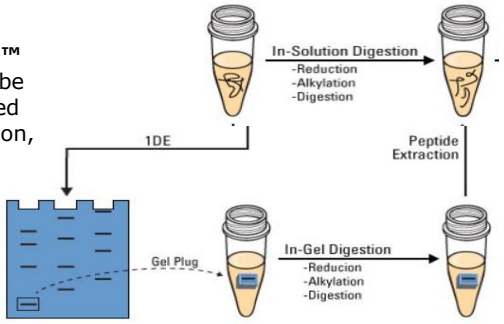
Enriched sub-proteomes remain on the bead and digested using Bead-Assisted Sample Prep (BASP); protocols provided with all **NRicher™** derived products



OR

Digest Options

Eluate from all **NRicher™** derived products ™ can be digested by any preferred method, In-Gel, In-Solution, FASP, etc.



LC-MS

Product	Size	Total RBC samples processed	Item No.
HemoVoid™ LC-MS On-Bead	10 Preps	10 x 100-200 µl samples	HVB-MS10

Items Required	10 Prep	Reagent
HemoVoid™ Beads	0.25 gram	Supplied
Binding Buffer HVBB (0.05M HEPES, pH 6.0)	6 ml	Supplied
Wash Buffer HVWB (0.05M HEPES, pH 7.0)	15 ml	Supplied
Spin-filter & tube assemblies	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.

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PROTOCOL

Based On Processing 100-200 µl Red Blood Cell (RBC) Lysate (compatible with RIPA buffer)

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. Depending upon the quality of the sample, centrifugation times can be adjusted to increase g's or time, sufficient to process the sample 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 1,000 g's. Discard the filtrate. Repeat step-1.
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 g's.
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 g's. 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 4 minutes at 5,000 g's, 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 4 minutes at 5,000 g's, and retain digested peptides filtrate.

11.To further extract remaining peptides, add 150 µL 10% formic acid, vortex 10 min, centrifuge at 4 minutes at 5,000 g's, 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.

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Selection of HemoVoid™ References:

Red Blood Cells (RBCs) / Parkinson's Disease / α-Synuclein

Cao, Chan, et al. " Deep learning-assisted single-molecule detection of protein post-translational modifications with a biological nanopore ." *bioRxiv* (2023): 2023-09. The authors demonstrate the ability of a biological nanopore, to detect and distinguish α-synuclein-derived peptides bearing single or multiple PTMs, occurring at different positions and in various combinations. To deplete Hemoglobin, the article states "Briefly, **RBCs ... treated using the HemoVoid** kit, … to remove hemoglobin but also to enrich low abundant proteins such as α-synuclein."

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.**

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".

Red Blood Cells (RBCs) / Other Applications

Mitra, Nibedita, et al. "Multi-Omics Analysis of Red Blood Cells Reveals Molecular Pathways Underlying Thalassemia Severity Beyond Globin Gene Mutations." *medRxiv* (2025): 2025-02.
The study aims were to identify dysregulated molecular pathways in red blood cells contributing to thalassemia severity. In the methods section for Sample Preparation for RBC Proteomics Study, the article states "hemoglobin was depleted using the **HemoVoid** kit…". This investigation finds six pathways which are responsible for thalassemia severity independent of mutational burden.

Pawliński, Łukasz, et al. "Proteomic biomarkers in Gaucher disease." *Journal of clinical pathology* 74.1 (2021): 25-29.
For proteomics, quantitative analysis was performed by the isobaric tag for a relative and absolute quantitation (iTRAQ) method. The article states "Cells were lysed in lysis buffer (7 mol/L urea, 2 mol/L thiourea, 4% CHAPS, 1% DTT …, vortexed, incubated at 25°C for 30 min and then centrifuged at 12 000×g for 15 min…the samples were purified using **HemoVoid** resin (Biotech Support Group) to remove haemoglobin contamination. The study found 31 proteins that significantly differed in concentration between GDt1 patients and a control group.

Wu, Na, et al. "Proteomic characteristics of plasma and blood cells in natural aging rhesus monkeys." *Proteomics*: 2200049.
This study sought to understand the aging process. For this purpose, the investigation analyzed and compared the protein expression spectrums in the blood of old and young rhesus monkeys. To extract blood cell proteins and deplete Hemoglobin, the article states "Blood cell proteins were lysed with…protein extraction solution (Bestbio, China)…After centrifugation…the supernatants were further **depleted of Hemoglobin using HemoVoid™**." Upon depletion, the study found 1183 proteins expressed differentially in blood cells.

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 speciesUsing 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.**

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Pawliński, Łukasz, et al. "Proteomic biomarkers in Gaucher disease." *Journal of clinical pathology* 74.1 (2021): 25-29. For proteomics, quantitative analysis was performed by the isobaric tag for a relative and absolute quantitation (iTRAQ) method. The article states "Cells were lysed in lysis buffer (7 mol/L urea, 2 mol/L thiourea, 4% CHAPS, 1% DTT …, vortexed, incubated at 25°C for 30 min and then centrifuged at 12 000×g for 15 min…the samples were purified using **HemoVoid** resin (Biotech Support Group) to remove haemoglobin contamination. The study found 31 proteins that significantly differed in concentration between GDt1 patients and a control group.

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

For Targeted Proteomics

NRicher™ Bead Platform Provides Unique Sub-Proteome Biases And Fit For Purpose Opportunities for Targeted LC-MS Quantification

Learn more at: https://www.biotechsupportgroup.com/category-s/335.htm

NRicher™ Beads Are Versatile to A Variety of Bead Processing Formats

In addition to standard spin-filter formats, other formats compatible with the 50 µm NRicher™ beads are:

High Throughput Automation Compatible INTip™ SPE (DPX Technologies) Format

The INTip™ SPE tip format improves ease of use and scalability to process multiple samples in parallel, utilizing 96-well plates and automated liquid handlers. The tip-based formats have been proven to be compatible with most automation platforms, i.e., Integra, Hamilton, etc. Please inquire for more information, as these formats are customized to the application and automation platform.

The NRicher™ beads can be readily processed in 96-well filter formats. Please inquire.

CONTACT US

We welcome your questions and comments regarding our products.

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