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HemoVoid™

Hemoglobin Depletion Plus Low Abundance Protein Enrichment For Erythrocyte Lysate Proteomics

- 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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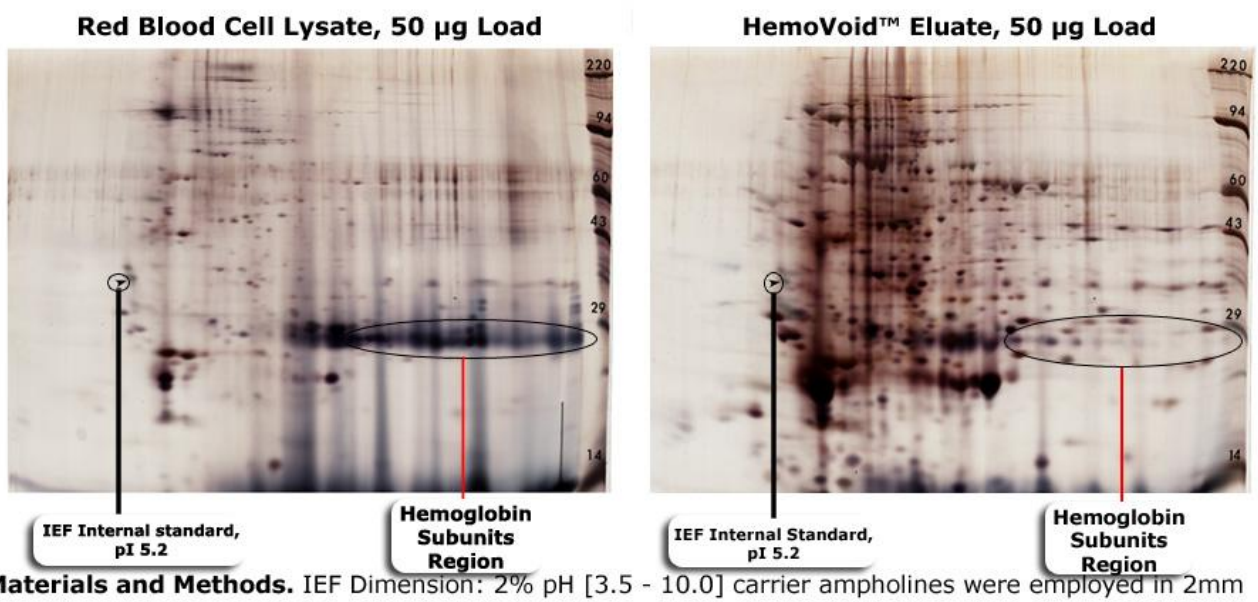

Materials and Methods. IEF Dimension: 2% pH [3.5 - 10.0] carrier ampholines were employed in 2mm glass tubes for focusing. Size dimension: Each IEF tube gel was sealed to a 10% acrylamide slab gel. After electrophoresis, proteins were fixed and silver stained. Molecular weight reference standards are represented on the far right side of each image.

Results and Discussion. When comparing the two gel images, the HemoVoid™ eluate (right) has been severely depleted of Hemoglobin. The remainder of the red cell proteins are substantially enriched (visualized) and are better resolved in the HemoVoid™ eluate. Many more proteins are detectable after HemoVoid™ treatment with extensive protein coverage across both dimensions.

Product	Size	Total samples processed	Item No.
HemoVoid™	10 Preps	10 x 300 µl	HVK-10
HemoVoid™	50 Preps	50 x 300 µl	HVK-50
HemoVoid™	100 Preps	100 x 300 µl	HVK-100
NOTE: Please contact sales@biotechsupportgroup.com **for prices in bulk quantities.**			

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Items Required	5 Prep (part of Catalog # HTK-05, HemoTrial Kit)	10 Prep	50 Prep	100 Prep	Reagent
HemoVoid™ beads	0.25 grams	0.5 grams	2.5 grams	5.0 grams	**Supplied**
Binding Buffer HVBB (0.05M HEPES, pH 6.0)	4 ml	8 ml	40 ml	80 ml	**Supplied**
Wash Buffer HVWB (0.05M HEPES, pH 7.0)	8 ml	15 ml	75 ml	150 ml	**Supplied**
Elution Buffer HVEB (0.25M Tris + 0.5M NaCl, pH 10.0-10.5)	2 ml	3 ml	15 ml	30 ml	**Supplied**
Spin-filter & tube assemblies	5	10	50	100	**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 300 µl Red Cell 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. Weigh out 50 mg of **HemoVoid™** beads in a spin-tube.

2. Add 250 µl of **Binding Buffer HVBB .**Vortex or mix well for 5 minutes at room temperature followed by centrifugation for 2 minutes at 1,000 g's. Discard the supernatant.

3. Repeat step-2

4. Add 300 µl of **HVBB** and 300 µl of the **Sample.** Vortex for 10 min and then centrifuge for 4 minutes at 5,000 g's.

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

6. To the pellet, add 500 µl of **Wash Buffer HVWB.** Vortex or mix well for 5 min and centrifuge for centrifuge for 4 minutes at 5,000 g's. Remove the filtrate as **Wash.**

7. Repeat Step-6, 2 times.

8. To the pellet, add 300 µl of **Elution Buffer HVEB.** Vortex or mix well for 10 min and centrifuge for centrifuge for 4 minutes at 5,000 g's. Remove the filtrate as **Elution.** The proteome eluate (0.25M Tris + 0.5M NaCl, pH 10.0-10.5) is ready for further functional, proteomic or LC-MS analysis.

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As an option for LC-MS sample preparation, the bead assisted on-bead digestion protocol (BASP™) is provided below. The digest buffer is not supplied but the Wash Buffer (0.05M HEPES, pH 7.0), can be made up, or comparable buffers (0.02-0.5M, pH 6-7) can be used. Higher pH buffers are not recommended.

6. Using **Wash Buffer**, prepare to 10mM of DTT concentration, and add 200 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 for 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** twice, to remove any traces of the filtrate.

9. Add 16 µg trypsin in 200 µl **Wash Buffer** 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 for 4 minutes at 5,000 g's, and retain digested peptides filtrate.

11.To further extract remaining peptides, add 300 µL 10% formic acid, vortex 10 min, centrifuge for 4 minutes at 5,000 g's., and combine this volume with volume from step 10.

12.Total is about 500µl. Prepare to desired final concentration. Store at -80°C until LC-MS/MS.

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".

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Vicente Miranda, Hugo, et al. "Posttranslational modifications of blood-derived alpha-synuclein as biochemical markers for Parkinson's disease." *Scientific reports* 7.1 (2017): 13713. Posttranslational modifications (PTMs) in aSyn have been identified and implicated on its pathobiology. Since aSyn is abundant in blood erythrocytes, the study aimed to evaluate whether PTMs of aSyn in the blood might hold value as a biomarker for PD. As haemoglobin is the major protein component of erythrocytes lysates (90%), the article states "we depleted this … **using HemoVoid…, enabling the additional concentration of other proteins of lower abundance. We confirmed the aSyn enrichment** by immunoblotting (SDS-PAGE and dot-blot)."

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.

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.

Das, Sonu, et al. "A journey to unravel the pathophysiology of stable and exacerbated Chronic obstructive pulmonary disease through erythrocyte proteomics: A combined mass spectrometry/bioinformatics approach." (2022). A label free relative quantification of erythrocyte cytosol proteome based on LC-MS/MS was performed on hemoglobin- depleted erythrocyte lysate samples of stable and exacerbated COPD, relative to healthy controls. To deplete Hemoglobin, the article states "**HemoVoid™, …** from erythrocyte lysate samples to unmask low abundance…proteins… ."

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.

Christer, M. A. L. M., et al. "Methods for the detection of autologous blood-doping." U.S. Patent Application No. 16/976,936.
The patent application relates to the detection of autologous blood doping. More specifically, the invention relates to methods comprising tryptic digestion of samples of isolated red blood cell (RBC), specifically isolated RBC cytosol, followed by peptide mapping using LC-MS/MS. The invention's description states "**Hemoglobin depletion was performed using HemoVoid resin**… .". Upon the preferable depletion of hemoglobin, the methods enable detection of increased levels of certain peptides in samples from subjects that have been subjected to autologous blood doping, compared to samples from non-doped control subjects.

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… .".

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

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(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**".

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 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™** 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
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 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.**

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

Red Blood Cells, Plasmodium extracts / Malaria
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).

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

Lan, Qin, and Zhao-bing Gu. "Data-independent acquisition-based proteome profiling of red blood cells from dairy buffaloes under different types of heat stress." *Veterinary and Animal Science* (2025): 100437. Heat stress (HS) induces hypoxia and oxidative stress, reducing animal health and livestock production. Red Blood Cell (RBC) lysates were isolated for data-independent acquisition-based proteomics to identify differentially expressed proteins involved in the HS response. The article states "**HemoVoid™ LC-MS On-Bead** kit (Biotech Support Group, HVB-MS10) was used to remove Hb". Digested peptides were analyzed by nanometric high performance liquid chromatography performed with a Q-Exactive HFX (Thermo Scientific) mass spectrometer, operated in DIA mode. Results showed that blood clotting factors, complement proteins, immunoglobulins, and vasoconstriction proteins were consistently decreased under the three types of HS conditions.

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. 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 article states "… 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…, following the manufacturer instructions".

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

CONTACT US

We welcome your questions and comments regarding our products.

Call 732-274-2866, 800-935-0628 (North America) Mon – Fri 9am-5pm EST.
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