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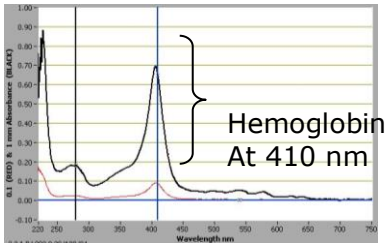
HemoVoid™ Hemoglobin Variant Enrichment From Blood

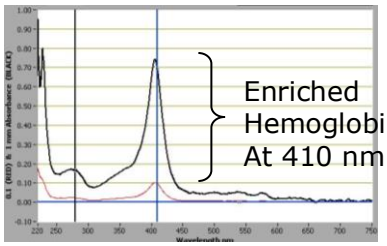
Purification & Enrichment Of Hemoglobin From Blood For Hemoglobin Variant Research

- Hemoglobin enrichment from fresh or frozen blood and dried blood spot/blood card etc.
- Enriched hemoglobin voids in flow-through >98% pure, with <30 minute bind/wash/elute protocol
- Disposable, cost-effective and high-throughput.
- Mild buffer condition maintains tertiary structure and simple transfer to secondary analysis
- Enriches hemoglobin from diverse species including human, sheep, mouse, goat, rat, etc.
- Enriched/purified Hemoglobin can be studied for variant research on Sickle cell disease, Thalassemia and other Hemoglobinopathies
- Eluted fractions contain hemoglobin depleted proteins suitable for all analytical platforms (i.e., LC-MS, Immunoassay)

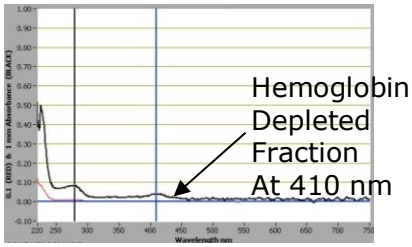
Hemoglobin Absorbance at 410 nm



Sheep Blood Load
(1:10 diluted)



Sheep Blood FT/Wash
(1:10 diluted)



Sheep Blood Elution

Product	Size	Blood sample processed	Item No.
HemoVoid™ **Hemoglobin Enrichment Kit**	10 Preps	500 µl of Blood Sample	HBV-10
HemoVoid™ **Hemoglobin Enrichment Kit**	50 Preps	2500 µl of Blood Sample	HBV-50
NOTE: Please contact sales@biotechsupportgroup.com for prices in bulk amount.			

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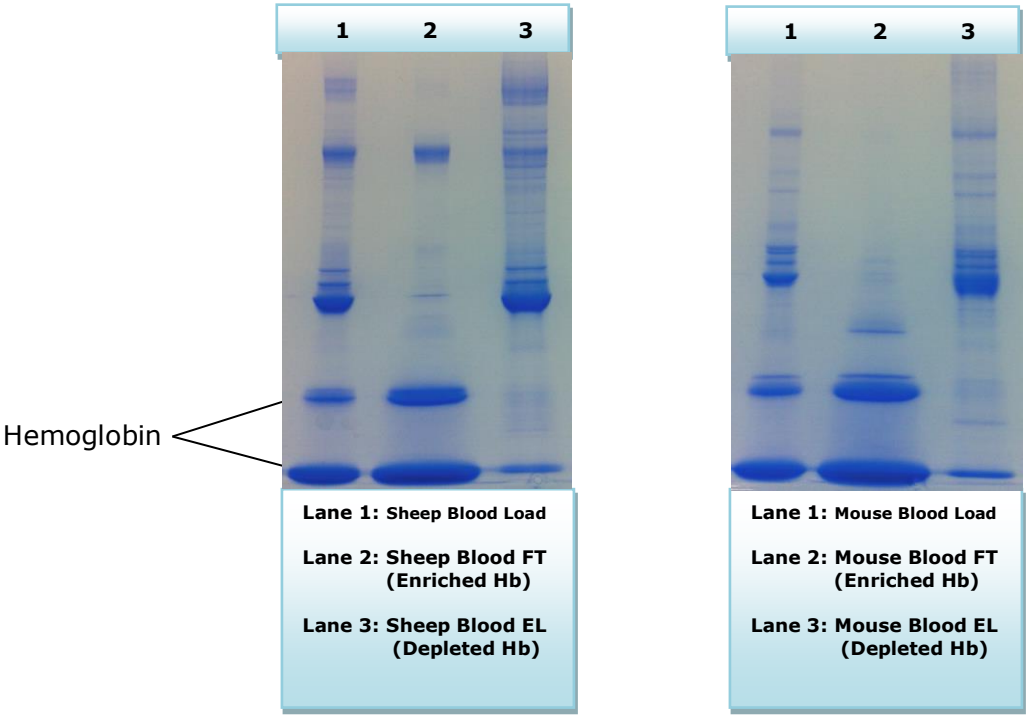
SDS-PAGE (4-20%), Left: Frozen Sheep Blood, Right: Frozen Mouse Blood



Items Required	10 Prep	50 Prep	Reagent
HemoVoid™ beads	0.5 gram	2.5 grams	**Supplied**
Binding Buffer HVBB (0.05M HEPES, pH 6.0)	12 ml	60 ml	**Supplied**
Wash Buffer HVWB (0.05M HEPES, pH 7.0)	3 ml	15 ml	**Supplied**
Elution Buffer HVEB (0.25M Tris + 0.5M NaCl, pH 9.0-10.0)	3 ml	15 ml	**Supplied**
Spin-filter & tube assemblies	10	50	**Supplied**

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

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Hemovoid™ Protocol For Hemoglobin Enrichment From Blood Samples For Hemoglobin Variant (HbS, HbE, HbC, HbD, HbF, HbA1c,Thalassemia, etc.) Research

Based On Processing 50 µl Blood (Anti-coagulated)

If using Red Blood Cell Lysate, please contact us for a modified protocol.

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. **SAMPLE LYSIS.** Add 300 µl of **Binding Buffer HVBB** and 50 µl of the **blood sample.** Vortex or mix thoroughly for 5 minutes at room temperature followed by centrifugation for 5 minutes at 5,000-10,000 rpm (2,000-8,000xg). Pipette the clarified supernatant and discard the pellet. At this stage, the sample lysate should be clear and free of colloidal material. If necessary, the lysate can be filtered through a 0.45 µm µm syringe, or microfuge-type filter before beginning the prep.
2. **BEAD CONDITIONING.** Weigh out 50 mg of **HemoVoid™** beads in a spin-filter. Add 250 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-2.
3. **SAMPLE PROCESSING.** Add 300 µl of **Binding Buffer HVBB** to **HemoVoid™** beads followed by 300 µl of the **Lysed Sample from Step 1** to the beads**.** Vortex or mix thoroughly for 10 min and then centrifuge for 4 minutes at 5,000-10,000 rpm (2,000-8,000xg).
4. To the pellet, add 300 µl of **Wash Buffer HVWB.** Vortex or mix thoroughly for 5 min and centrifuge for 2 minutes at 5000 rpm. Remove the filtrate as **Wash. The combined filtrates from steps 3 & 4** contain the enriched hemoglobin (red color) and is ready for **hemoglobin variant analysis.**
5. **BEAD ELUTION.** To the beads, add 300 µl of **Elution Buffer HVEB.** Vortex or mix thoroughly for 10 min and centrifuge for 2 minutes at 5000 rpm. Remove this filtrate as the **Hemoglobin-depleted sub-proteome** (0.25M Tris + 0.5M NaCl, pH 9.0-10.0), suitable for further analysis.

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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. Also, there were decreased levels of three key enzymes in glutathione metabolism in VFEpoR RBC.

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

Immediately upon implantation, scaffolds for bone repair are exposed to the patient's blood. Biphasic calcium phosphate (BCP) ceramics are considered as the gold standard in bone reconstruction surgery. Here, 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]. They were let on ice for 30min with regular vortexings and centrifuged at 4°C, 8000g, 15 min. 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.

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.".

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.

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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

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.

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.

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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)".

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)
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".

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

Related Separations, Enrichment/Depletion & Sample Prep - All Product Categories (https://www.biotechsupportgroup.com/Products-a-z_a/258.htm)

Albumin & IgG Removal (https://www.biotechsupportgroup.com/Articles.asp?ID=451)

Lipid Removal and Clarification (https://www.biotechsupportgroup.com/Articles.asp?ID=456)

Hemoglobin Removal (https://www.biotechsupportgroup.com/Articles.asp?ID=452)

Sample Prep – Liquid Biopsy (https://www.biotechsupportgroup.com/Articles.asp?ID=457)

Sample Prep – Glyco, Virus, Kinase, Aqueous Protein Crash/Metabolomics (https://www.biotechsupportgroup.com/Articles.asp?ID=453)

Sample Prep – Mass Spectrometry (https://www.biotechsupportgroup.com/Articles.asp?ID=432)

Sample Prep – Genomics (https://www.biotechsupportgroup.com/Articles.asp?ID=455)

CONTACT US

We welcome your questions and comments regarding our products.

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