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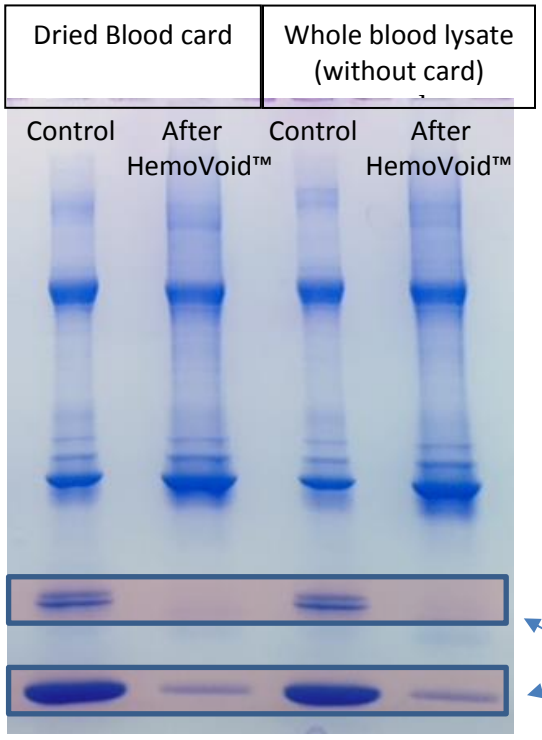
HemoVoid™ Blood Card Kit

Hemoglobin Depletion And Protein Enrichment From Dried Whole Blood Cards

- Dried blood spots are useful for low volume analyses such as for neonatal testing
- Protocols suitable for inexpensive blood card systems, no need for cell separation
- Hemoglobin voids in flow-through >98%, with <30 minute bind/wash/elute protocol
- Hemoglobin removal from whole blood lysates extracted from dried blood cards
- Blood proteins and enzymes are enriched for biomarker and proteomic investigations.
- Removes hemoglobin from diverse species incl. human, sheep, bovine, goat, rat, mouse, etc.

Hemoglobin is a common contaminant from dried whole blood cards and not normally found in serum samples. The **HemoVoid™** Blood Card protocol was designed to substantially reduce the presence of hemoglobin and its associated interference with many serum protein analytes.

HemoVoid™, is derived from **NuGel™** silica-based mixed mode beads, and selectively voids out (negative selection) hemoglobin from dried whole blood cards, enriching the remaining proteome on the beads. The **HemoVoid™** protocol uses mild buffers; the protocol conditions are gentle so that native enzyme activity is retained in elution fractions.



	From Dried Blood Card	Whole blood lysate (without card)
Sample	**HemoVoid™ Yield (µg)**	**HemoVoid™ Yield (µg)**
10 µl of sheep blood	250	230

The **HemoVoid™ Blood Card** protocol reduces the hemoglobin concentration, enriching the remaining blood proteome with equivalent yield to **HemoVoid™** separation, without first drying whole blood on a card.

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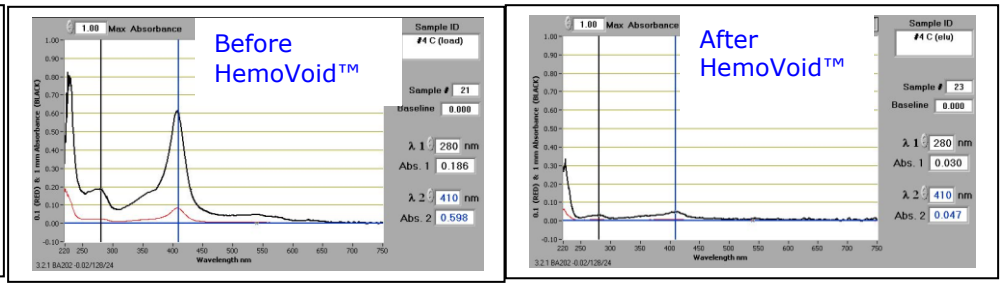
Abs at 410 nm shows presence of hemoglobin. On left, proteins extracted from dried blood card show high hemoglobin concentration. On Right, after HemoVoid™ treatment, hemoglobin is severely depleted.



Product	# of samples processed	Item No.
HemoVoid™ Blood Card	10 Dried Whole Blood Card 0.5" Spots	HVBC-10
HemoVoid™ Blood Card	50 Dried Whole Blood Card 0.5" Spots	HVBC-50
NOTE: Please contact sales@biotechsupportgroup.com for prices in bulk amount.		

Product	# of samples processed	Item No.
Hemoglobin Removal Blood Card Trial Kit	5 Preps each HemoVoid™ & HemogloBind, each to process 0.5" Dried Blood Spots	HRBC-05

Kit Content	5 Prep	10 Prep	50 Prep	Reagent
HemoVoid™ Beads	0.25 gram	0.5 gram	2.5 grams	**Supplied**
Protein Extraction Buffer PEB	2.5 ml	5 ml	25 ml	**Supplied**
Binding Buffer HVBB, *PH 6.0*	8 ml	15 ml	75 ml	**Supplied**
Wash Buffer HVWB, *PH 7.0*	8 ml	15 ml	75 ml	**Supplied**
Elution Buffer HVEB, *PH 9.8*	2 ml	3 ml	15 ml	**Supplied**
SpinX Centrifuge tube filters	5	10	50	**Supplied**
Suggested Or Equivalent Supplier of Blood Card: Whatman 903™ Protein Saver cards				**Not Supplied**

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HemoVoid™ Protocol For Hemoglobin Depletion From Blood Spot/Blood Card

Based on processing 10-20 µl whole blood applied to and dried on Whatman 903™ Protein Saver cards

1. **Extraction of dried protein from the card.** Punch out the dried blood section from the card into a microfuge tube. Add 400 µl **PEB** buffer. Shake for 30 minutes at room temperature. Centrifuge at 5000 rpm for 4 minutes. This is the Sample used for Step #5.

2. Weigh out 50 mg of **HemoVoid™** matrix into the supplied SpinX filter.

3. Add 400 µl of **Binding Buffer HVBB** to the SpinX filter. Vortex or mix well for 5 minutes at room temperature followed by centrifugation at 3000 rpm. Discard the supernatant.

4. Repeat step 3.

5. Add 200 µl of **Binding Buffer HVBB** to the SpinX filter. Add 300 µl of the Sample prepared in step 1 to the same SpinX filter. Vortex for 10 min and then centrifuge for 2 minutes at 5000 rpm.

6. Discard the hemoglobin containing filtrate.

7. To the pellet, add 500 µl of **Wash Buffer HVWB**. Vortex or mix well for 5 min and centrifuge for 2 minutes at 5000 rpm. Discard the filtrate.

8. Repeat Step 7, twice.

9. To the pellet, add 200 µl of **Elution Buffer HVEB**. Vortex or mix well for 10 min and centrifuge for 2 minutes at 5000 rpm. Analyze the hemoglobin depleted eluate proteome.

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

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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.

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.

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;

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We welcome your questions, comments and concerns regarding our products.

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