



## BIOTECH SUPPORT GROUP

### 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.

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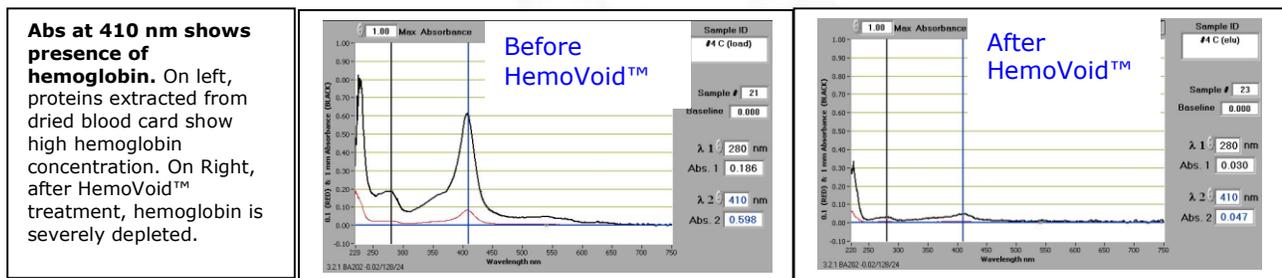
Sample	From Dried Blood Card HemoVoid™ Yield (µg)	Whole blood lysate (without card) HemoVoid™ Yield (µg)
10 µl of sheep blood	250	230

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.

Hemoglobin subunit regions



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Product	# of samples processed	Item No.
<b>HemoVoid™ Blood Card</b>	10 Dried Whole Blood Card 0.5" Spots	HVBC-10
<b>HemoVoid™ Blood Card</b>	50 Dried Whole Blood Card 0.5" Spots	HVBC-50
<b>NOTE: Please contact <a href="mailto:sales@biotechsupportgroup.com">sales@biotechsupportgroup.com</a> for prices in bulk amount.</b>		
Product	# of samples processed	Item No.
<b>Hemoglobin Removal Blood Card Trial Kit</b>	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	<b>Supplied</b>
Protein Extraction Buffer PEB	2.5 ml	5 ml	25 ml	<b>Supplied</b>
Binding Buffer HVBB, PH 6.0	8 ml	15 ml	75 ml	<b>Supplied</b>
Wash Buffer HVWB, PH 7.0	8 ml	15 ml	75 ml	<b>Supplied</b>
Elution Buffer HVEB, PH 9.8	2 ml	3 ml	15 ml	<b>Supplied</b>
SpinX Centrifuge tube filters	5	10	50	<b>Supplied</b>
Suggested Or Equivalent Supplier of Blood Card: Whatman 903™ Protein Saver cards				<b>Not Supplied</b>



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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.

### Related HemoVoid™ References

#### Human Red Blood Cells (RBC)

[HemoVoid™ On Bead Digestion Application Work On RBC](#) by Irene Granlund, *Umeå University*

#### 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.



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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;

### **Red Blood Cell Lysate**

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.

Lange, Philipp F., Pitter F. Huesgen, Karen Nguyen, and Christopher M. Overall. "[Annotating N termini for the Human Proteome Project: N termini and N \$\alpha\$ -acetylation status differentiate stable cleaved protein species from degradation remnants in the human erythrocyte proteome.](#)" *Journal of proteome research* (2014).

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

## CONTACT US

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