BIOTECH SUPPORT GROUP

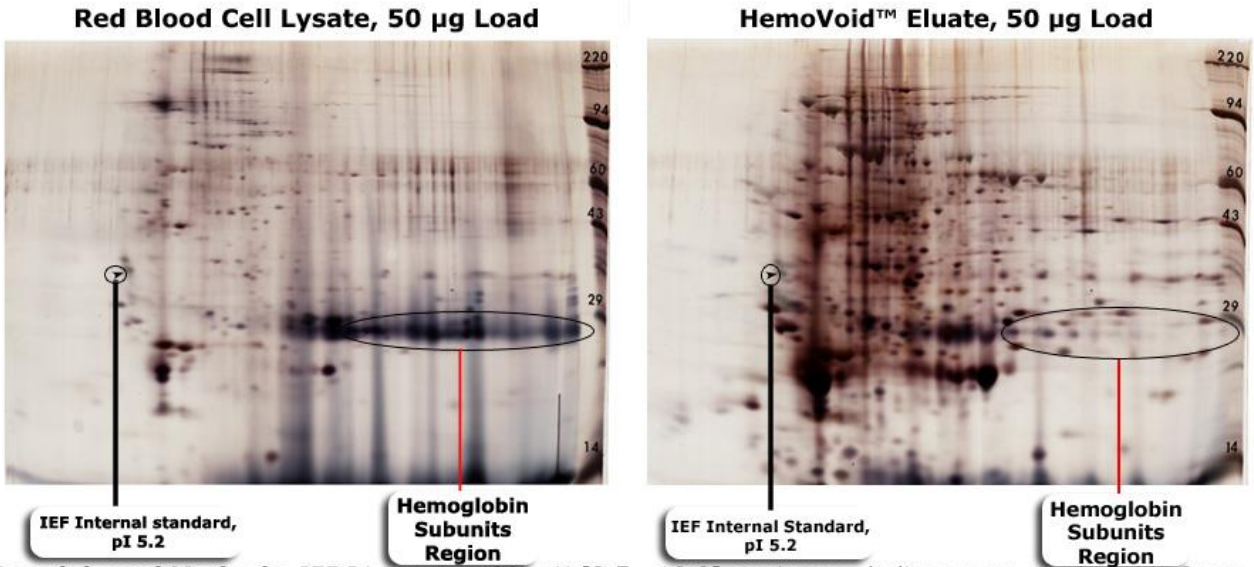
HemoVoid™ Hemoglobin Depletion Kit

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 hemolyzed serum
- Low abundance protein and enzyme enrichment
- Disposable, cost-effective
- Mild elution maintains tertiary structure and simple transfer to secondary analysis
- Removes hemoglobin from species including human, sheep, bovine, goat, etc.
- The eluted fractions retain their enzymatic and biological activity

HemoVoid™, a silica-based protein enrichment matrix, removes hemoglobin from erythrocyte lysate samples while concentrating low abundance, and/or low molecular weight proteins. The **HemoVoid™** protocol uses mild buffers; the protocol conditions are so gentle that native enzyme activity is retained in elution fractions.

HemoVoid™ derives from a silica-based library of individual mixed-mode ligand combinations (ionic, hydrophobic, aromatic, polymer). The library was designed to facilitate weak binding of proteins, allowing for rapid elution from the matrix without any foreknowledge of the variety of proteins contained in the starting sample. **HemoVoid™** depletes hemoglobin from red cell lysates while enriching the less abundant blood proteins.



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.

BIOTECH SUPPORT GROUP

Product	Size	Total samples processed	Item No.	Price
HemoVoid™	5 Preps	5 x 300 µl	HVK-05	$185
HemoVoid™	10 Preps	10 x 300 µl	HVK-10	$365
HemoVoid™	50 Preps	50 x 300 µl	HVK-50	$1375
HemoVoid™	100 Preps	100 x 300 µl	HVK-100	$2175
NOTE: Please contact sales@biotechsupportgroup.com **for prices in bulk quantities.**				

Items Required	5 Prep	10 Prep	50 Prep	Reagent
HemoVoid™	0.25 gram	0.5 gram	2.5 grams	**Supplied**
Binding Buffer HVBB, *PH 6.0*	6 ml	15 ml	125 ml	**Supplied**
Wash Buffer HVWB, *PH 7.0*	6 ml	15 ml	125 ml	**Supplied**
Elution Buffer HVEB, *PH 9.8*	6 ml	15 ml	125 ml	**Supplied**
SpinX Centrifuge tube filters	5	10	50	**Supplied**

PROTOCOL – Based on processing 300 µl Sample

1. Weigh out 50 mg of **HemoVoid™** matrix 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 3000 rpm. 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 10,000 rpm.

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 4 minutes at 10000 rpm. Remove the filtrate as **Wash.**

7. Repeat Step-6, 2 times. **The bead is now enriched with hemoglobin depleted proteins. For LC-MS sample preparation, an on-bead digestion protocol can be applied (protocol follows on next page). Otherwise proceed to the next step.**

8. To the pellet, add 300 µl of **Elution Buffer HVEB.** Vortex or mix well for 10 min and centrifuge for 4 minutes at 10,000 rpm. Remove the filtrate as **Elution.**

9. The eluate is ready for further functional or LC–MS studies.

Note:

- The protocol can be scaled up or down proportionally to adjust for different serum volumes. The surface amount can be adjusted to accommodate more or less hemoglobin removal.
- We have 0.45µ SpinX centrifuge tube filters. If required can be ordered separately.

BIOTECH SUPPORT GROUP

Suggested On-Bead Digestion Protocol

- After the final wash steps from step 7, add 100 µls of 10 mM DTT solution to the beads for complete immersion, mix and incubate at 60°C for ½ hour.
- After cooling, add 100 µls of 50 mM iodoacetamide to the DTT/bead suspension, mix and incubate in the dark for 1 hour.
- Centrifuge at 5000xg (medium setting, not max) for 3 mins, and discard supernatant.
- On-bead digestion is done by adding 200 µls of a 0.125 ug/uL (or calculated to a user preferred ratio – typically 50-100:1 w:w, protein:trypsin) of MS-grade Trypsin to the beads. Digest overnight at 37°C.
- Centrifuge at 5000xg (medium setting, not max) for 3 mins, and retain peptide filtrate.
- To further extract remaining peptides, add 200 µls of 10% solution of formic acid to the beads.
- Incubate for 15 minutes at 37°C, centrifuge at 5000xg (medium setting, not max) for 3 mins, and add this volume to the first volume.
- Reduce to final volume using a SpeedVac.

References

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;

Red Blood Cell Lysate
Lange, Philipp F., 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." *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

BIOTECH SUPPORT GROUP

CONTACT US

We welcome your questions and comments regarding our products.

Tel: 732-274-2866, 800-935-0628 (North America) Mon – Fri 9am-6pm EST.

Fax 732-274-2899

Email sales@biotechsupportgroup.com

Mail 1 Deer Park Drive, Suite M, Monmouth Junction, NJ 08852

Web: www.biotechsupportgroup.com