**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 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.

<table>
<thead>
<tr>
<th>Product</th>
<th>Size</th>
<th>Total samples processed</th>
<th>Item No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>HemoVoid™</td>
<td>10 Preps</td>
<td>10 x 300 µl</td>
<td>HVK-10</td>
</tr>
<tr>
<td>HemoVoid™</td>
<td>50 Preps</td>
<td>50 x 300 µl</td>
<td>HVK-50</td>
</tr>
<tr>
<td>HemoVoid™</td>
<td>100 Preps</td>
<td>100 x 300 µl</td>
<td>HVK-100</td>
</tr>
</tbody>
</table>

NOTE: Please contact sales@biotechsupportgroup.com for prices in bulk quantities.
PROTOCOL – Based on processing 300 µl Sample

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.

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.

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

Note:

- Download HemoVoid™ LC-MS On-Bead Trypsin Digestion Protocol
- 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.

<table>
<thead>
<tr>
<th>Items Required</th>
<th>10 Prep</th>
<th>50 Prep</th>
<th>100 Prep</th>
<th>Reagent</th>
</tr>
</thead>
<tbody>
<tr>
<td>HemoVoid™</td>
<td>0.5 gram</td>
<td>2.5 grams</td>
<td>5.0 grams</td>
<td>Supplied</td>
</tr>
<tr>
<td>Binding Buffer HVBB, PH 6.0</td>
<td>8 ml</td>
<td>40 ml</td>
<td>80 ml</td>
<td>Supplied</td>
</tr>
<tr>
<td>Wash Buffer HVWB, PH 7.0</td>
<td>15 ml</td>
<td>75 ml</td>
<td>150 ml</td>
<td>Supplied</td>
</tr>
<tr>
<td>Elution Buffer HVEB, PH 9.8</td>
<td>3 ml</td>
<td>15 ml</td>
<td>30 ml</td>
<td>Supplied</td>
</tr>
<tr>
<td>SpinX Centrifuge tube filters</td>
<td>10</td>
<td>50</td>
<td>100</td>
<td>Supplied</td>
</tr>
</tbody>
</table>
References

Human Red Blood Cells (RBC)
HemoVoid™ On Bead Digestion Application Work On RBC by Irene Granlund, Umeå University

Red Blood Cells, Plasmodium extracts

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

Red Blood Cell Lysate


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;


CONTACT US
We welcome your questions and comments regarding our products.

Call 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