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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.

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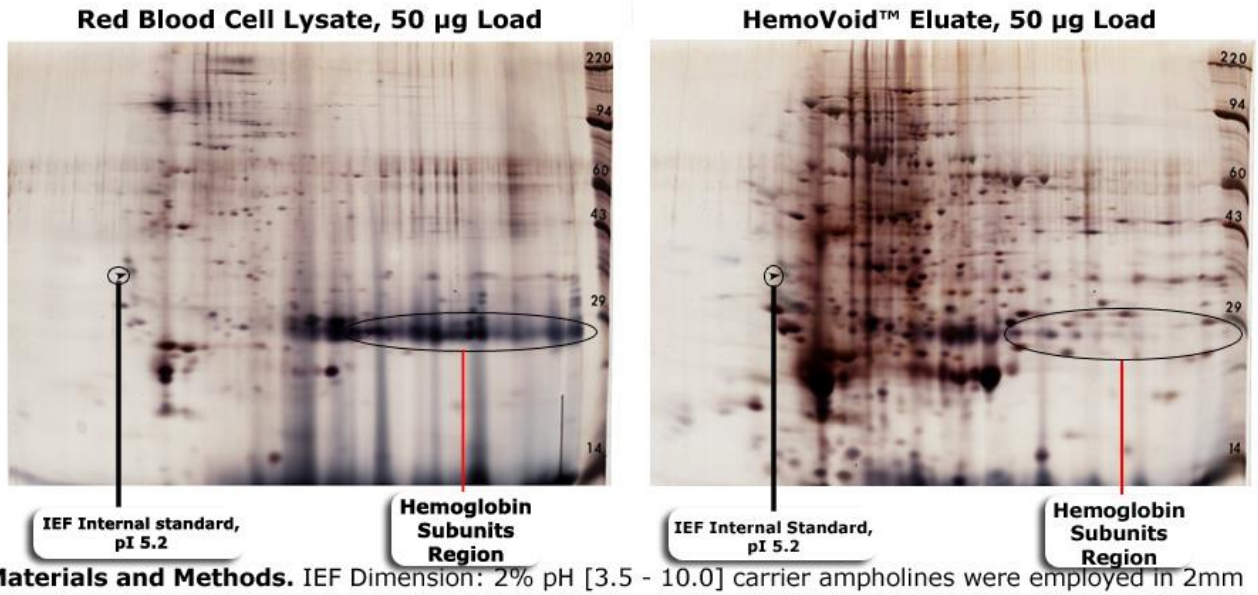

Materials and Methods. IEF Dimension: 2% pH [3.5 - 10.0] carrier ampholines were employed in 2mm glass tubes for focusing. Size dimension: Each IEF tube gel was sealed to a 10% acrylamide slab gel. After electrophoresis, proteins were fixed and silver stained. Molecular weight reference standards are 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.

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

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Items Required	10 Prep	50 Prep	100 Prep	Reagent
HemoVoid™	0.5 gram	2.5 grams	5.0 grams	**Supplied**
Binding Buffer HVBB, *PH 6.0*	8 ml	40 ml	80 ml	**Supplied**
Wash Buffer HVWB, *PH 7.0*	15 ml	75 ml	150 ml	**Supplied**
Elution Buffer HVEB, *PH 9.8*	3 ml	15 ml	30 ml	**Supplied**
SpinX Centrifuge tube filters	10	50	100	**Supplied**

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.

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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

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