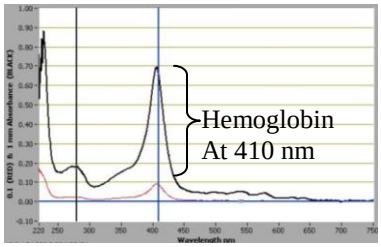
DATA SHEET

HemoVoid™ Hemoglobin Enrichment Kit

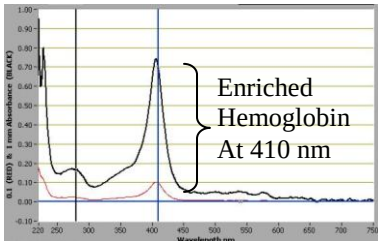
Purification & Enrichment Of Hemoglobin From Blood For Hemoglobin Variant Research

- Hemoglobin enrichment from fresh or frozen blood and dried blood spot/blood card etc.
- Enriched hemoglobin voids in flow-through >98% pure, with <30 minute bind/wash/elute protocol
- Disposable, cost-effective and high-throughput.
- Mild buffer condition maintains tertiary structure and simple transfer to secondary analysis
- Enriches hemoglobin from diverse species including human, sheep, mouse, goat, rat, etc.
- Enriched/purified hemoglobin can be studied for variant research and other research applications.
- Eluted fractions contains hemoglobin depleted proteins which can be used for LC-MS, proteomic studies

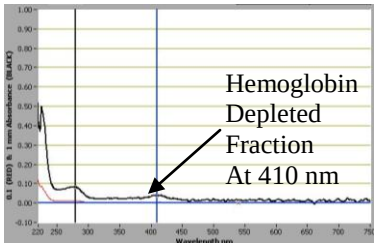
Hemoglobin Absorbance at 410 nm



Sheep Blood Load
(1:10 diluted)

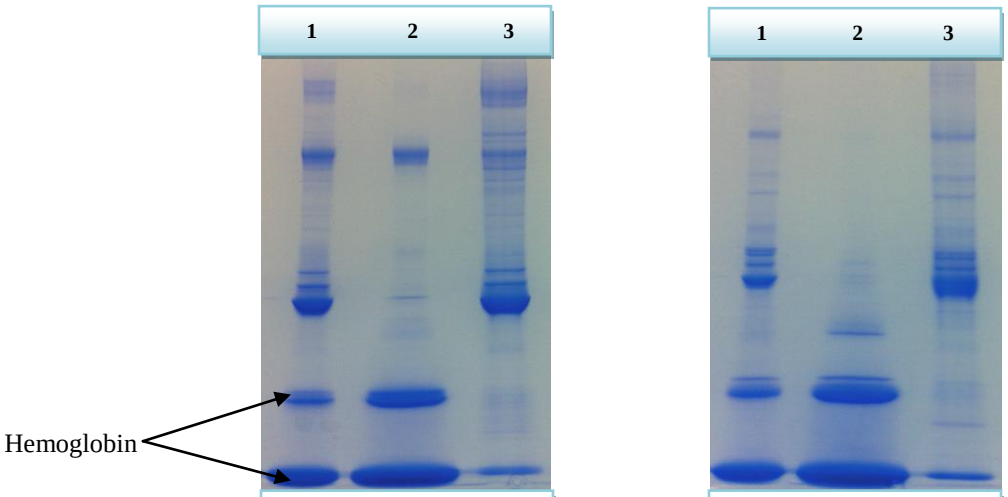


Sheep Blood FT/Wash
(1:10 diluted)



Sheep Blood Elution

SDS-PAGE (4-20%), Left: Frozen Sheep Blood, Right: Frozen Mouse Blood



Items Required	10 Prep	50 Prep	Reagent
HemoVoid™	0.5 gram	2.5 grams	**Supplied**
Binding Buffer HVBB, *PH 6.0*	12 ml	60 ml	**Supplied**
Wash Buffer HVWB, *PH 7.0*	12 ml	60 ml	**Supplied**
Elution Buffer HVEB, *PH 9.8*	12 ml	60 ml	**Supplied**
SpinX Centrifuge tube filters	10	50	**Supplied**

Hemovoid™ Protocol For Hemoglobin Enrichment From Blood Samples For Hemoglobin Variant (HbS, HbE, HbC, HbD, HbF, HbA1c,Thalassemia, etc.) Research

Based on processing 30 µl Sample

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

2. Add 300 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.

3. Repeat step-2

4. Add 300 µl of **HVBB** and 30 µl of the **Sample.** Vortex for 10 min and then centrifuge for 2 minutes at 5000 rpm. Pippet off the supernatant and discard the pellet.

5. Add the supernatant (step 4) to the equilibrated surface (step 3). Vortex for 10 min and then centrifuge for 2 minutes at 5000 rpm. Remove the filtrate as Flow-Through **FT** which contains enriched hemoglobin and is ready for further analysis.

   Note: If using RBC Lysate, add additional **Binding Buffer HVBB** (1:1 ratio of RBC Lysate to HVBB). Then continue from Step 4.

6. To the pellet, add 300 µl of **Wash Buffer HVWB.** Vortex or mix well for 5 min and centrifuge for 2 minutes at 5000 rpm. Remove the filtrate as **Wash** which contains residual enriched hemoglobin and is ready for **hemoglobin variant** analysis. Note**:** If necessary, Wash and Flow-Through can be mixed.

7. To the pellet, add 300 µl of **Elution Buffer HVEB.** Vortex or mix well for 10 min and centrifuge for 2 minutes at 5000 rpm. Remove this filtrate as **Hemoglobin depleted blood protein**. The elution contains hemoglobin depleted protein. This elution is now ready for further analysis.

8. **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.

Related HemoVoid™ References

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;

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