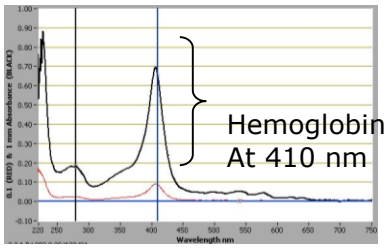
BIOTECH SUPPORT GROUP

HemoVoid™ Hemoglobin Variant Enrichment From Blood

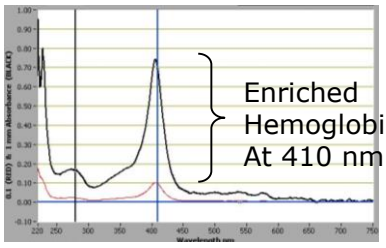
Purification & Enrichment Of Hemoglobin From Blood For Hemoglobin Variant Research

- 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

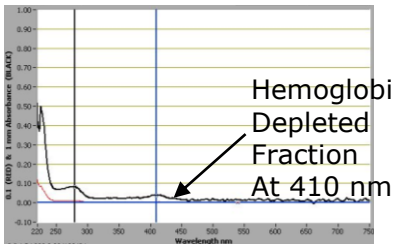
Hemoglobin Absorbance at 410 nm



Sheep Blood Load
(1:10 diluted)



Sheep Blood FT/Wash
(1:10 diluted)



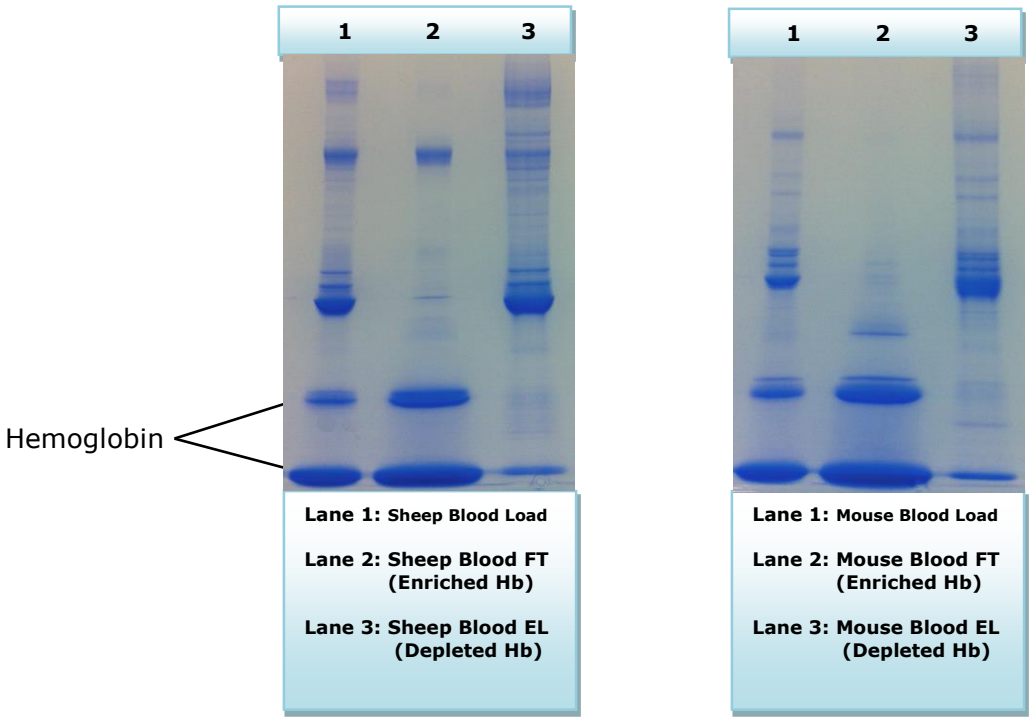
Sheep Blood Elution

Product	Size	Blood sample processed	Item No.
HemoVoid™ **Hemoglobin Enrichment Kit**	10 Preps	500 µl of Blood Sample	HBV-10
HemoVoid™ **Hemoglobin Enrichment Kit**	50 Preps	2500 µl of Blood Sample	HBV-50
NOTE: Please contact sales@biotechsupportgroup.com for prices in bulk amount.			

Gentaur Europe BVBA Voortstraat 49, 1910 Kampenhout BELGIUM
Tel 0032 16 58 90 45 info@gentaur.com

BIOTECH **SUPPORT** GROUP

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*	3 ml	15 ml	**Supplied**
Elution Buffer HVEB, *PH 9.8*	3 ml	15 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 50 µl Blood 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 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 50 µl of the **blood 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

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.

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 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: 0032 16 58 90 45
Email: info@gentaur.com