05152025

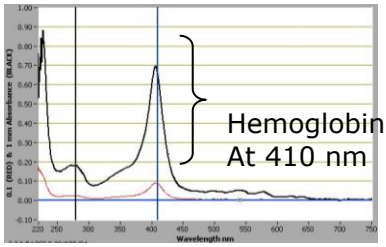
BIOTECH SUPPORT GROUP

HemoVoid™ Hemoglobin Variant Enrichment From Blood

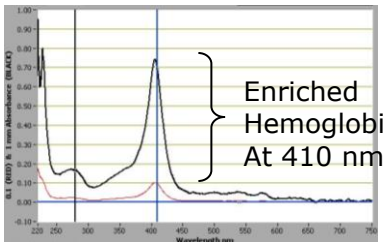
Purification & Enrichment Of Hemoglobin From Blood For Hemoglobin Variant Research

- 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 on Sickle cell disease, Thalassemia and other Hemoglobinopathies
- Eluted fractions contain hemoglobin depleted proteins suitable for all analytical platforms (i.e., LC-MS, Immunoassay)

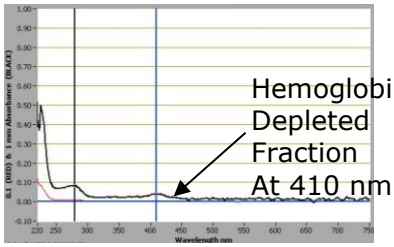
Hemoglobin Absorbance at 410 nm



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.			

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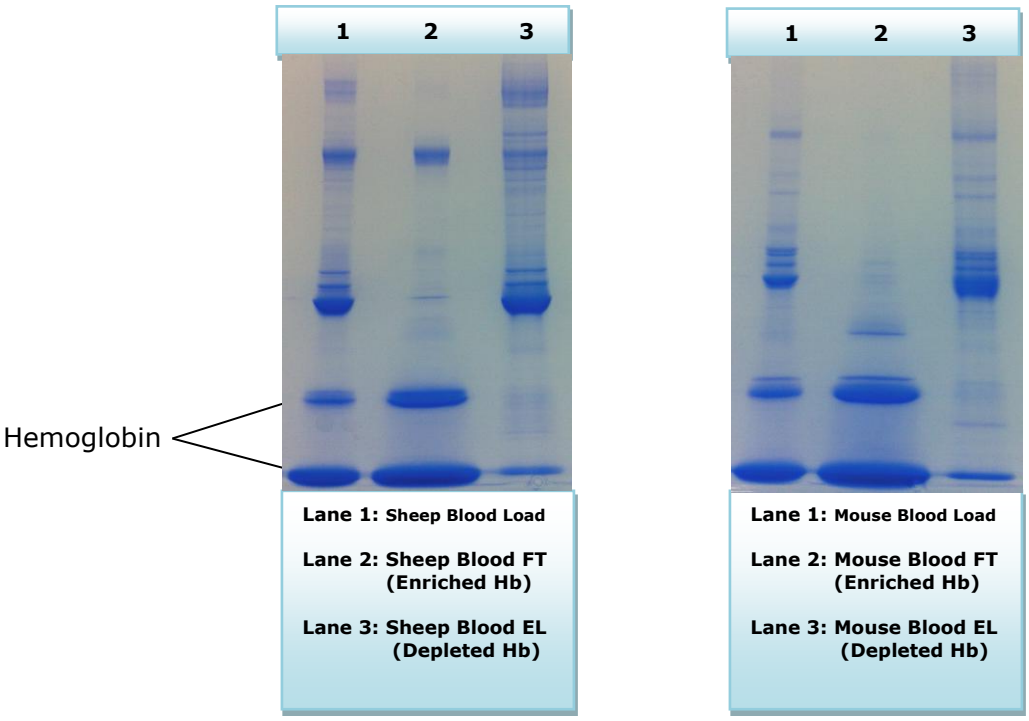
SDS-PAGE (4-20%), Left: Frozen Sheep Blood, Right: Frozen Mouse Blood



Items Required	10 Prep	50 Prep	Reagent
HemoVoid™ beads	0.5 gram	2.5 grams	**Supplied**
Binding Buffer HVBB (0.05M HEPES, pH 6.0)	12 ml	60 ml	**Supplied**
Wash Buffer HVWB (0.05M HEPES, pH 7.0)	3 ml	15 ml	**Supplied**
Elution Buffer HVEB (0.25M Tris + 0.5M NaCl, pH 9.0-10.0)	3 ml	15 ml	**Supplied**
Spin-filter & tube assemblies	10	50	**Supplied**

Additional Spin-Filters (low protein binding, 0.45 µm filter element) can be purchased separately, please inquire.

If there are any questions about compatibility or substitution with other buffers, please contact us.

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

Based On Processing 50 µl Blood (Anti-coagulated) (compatible with RIPA buffer)

If using Red Blood Cell Lysate, please contact us for a modified protocol.

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. Depending upon the quality of the sample, centrifugation times can be adjusted to increase g's or time, sufficient to process the sample through the beads.

The protocol can be scaled up or down proportionally to adjust for different volumes. The bead amount can be adjusted to accommodate more or less Hemoglobin removal.

1. **SAMPLE LYSIS.** Add 300 µl of **Binding Buffer HVBB** and 50 µl of the **blood sample.** Vortex or mix thoroughly for 5 minutes at room temperature followed by centrifugation for 5 minutes at 5,000-10,000 rpm (2,000-8,000xg). Pipette the clarified supernatant and discard the pellet. At this stage, the sample lysate should be clear and free of colloidal material (i.e., membrane "ghosts"). If necessary, the lysate can be clarified through a 0.45 µm µm microfuge-type filter before beginning the prep.
2. **BEAD CONDITIONING.** Weigh out 50 mg of **HemoVoid™** beads in a spin-filter. Add 250 µl of **Binding Buffer HVBB.** Vortex or mix thoroughly for 5 minutes at room temperature followed by centrifugation for 2 minutes at 1,000 g's. Discard the filtrate. Repeat step-2.
3. **SAMPLE PROCESSING.** Add 300 µl of **Binding Buffer HVBB** to **HemoVoid™** beads followed by 300 µl of the **Lysed Sample from Step 1** to the beads**.** Vortex or mix thoroughly for 10 min and then centrifuge for 4 minutes at 5,000 g's.
4. To the pellet, add 300 µl of **Wash Buffer HVWB.** Vortex or mix thoroughly for 5 min and centrifuge for 4 minutes at 5,000 g's. Remove the filtrate as **Wash. The combined filtrates from steps 3 & 4** contain the enriched hemoglobin (red color) and is ready for **hemoglobin variant analysis.**
5. **BEAD ELUTION.** To the beads, add 300 µl of **Elution Buffer HVEB.** Vortex or mix thoroughly for 10 min and centrifuge for 4 minutes at 5,000 g's. Remove this filtrate as the **Hemoglobin-depleted sub-proteome** (0.25M Tris + 0.5M NaCl, pH 9.0-10.0), suitable for further analysis.

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Selection of HemoVoid™ References:

Red Blood Cells (RBCs) / Parkinson's Disease / α-Synuclein

Cao, Chan, et al. " Deep learning-assisted single-molecule detection of protein post-translational modifications with a biological nanopore ." *bioRxiv* (2023): 2023-09. The authors demonstrate the ability of a biological nanopore, to detect and distinguish α-synuclein-derived peptides bearing single or multiple PTMs, occurring at different positions and in various combinations. To deplete Hemoglobin, the article states "Briefly, **RBCs ... treated using the HemoVoid** kit, … to remove hemoglobin but also to enrich low abundant proteins such as α-synuclein."

Klatt, Stephan, et al. "Optimizing red blood cell protein extraction for biomarker quantitation with mass spectrometry." *Analytical and Bioanalytical Chemistry* (2020): 1-14.
The article describes the advantage of **HemoVoid™** in detection of low abundance proteins when comparing their amounts (in percent) between four alternative extraction conditions, stating "… Most peptides, following **HemoVoid™** extraction, showed ion abundances ranging between 1.00E+5 and 1.00E+6 (31%). In comparison to this, fewer peptides (10–23%) were within this range following extraction with all other protocols". With respect to potential biomarkers for Parkinson's Disease, the article states "For example, PRDX6 accounts for 0.4% of the total ion abundance after DOC (deoxycholate) extraction, whereas following HV (**HemoVoid™**) extraction, this increases to 8%, a 20-fold enrichment". **The authors conclude that the HemoVoid™ method significantly reduces the concentration of hemoglobin, resulting in an increased signal-to noise of the remaining red cell proteins.**

Elhadi, Suaad Abd, et al. "α-Synuclein in blood cells differentiates Parkinson's disease from healthy controls." *Annals of Clinical and Translational Neurology*. The goal of this study was to determine whether blood cells expressing α-Synuclein can differentiate Parkinson's disease (PD) from healthy controls. Two proteoforms - PSer129 a-Syn (phosphorylated pathological form in Lewy bodies) and Oxidized a-Syn levels are observed in blood cells, but both at considerably lower concentration than total a-Syn, so the extremely high abundance of hemoglobin interferes with their analysis. To compensate, the article states for PSer129 α -Syn & Oxidized α -Syn detection by immunoassay, "**followed from hemoglobin clearance with HemoVoid** kit".

Red Blood Cells (RBCs) / Other Applications
Mitra, Nibedita, et al. "Multi-Omics Analysis of Red Blood Cells Reveals Molecular Pathways Underlying Thalassemia Severity Beyond Globin Gene Mutations." *medRxiv* (2025): 2025-02.
The study aims were to identify dysregulated molecular pathways in red blood cells contributing to thalassemia severity. In the methods section for Sample Preparation for RBC Proteomics Study, the article states "hemoglobin was depleted using the **HemoVoid** kit…". This investigation finds six pathways which are responsible for thalassemia severity independent of mutational burden.

For a full list of Hemoglobin Removal references, visit:
https://www.biotechsupportgroup.com/References-s/138.htm#hemoglobin-depletion

For Targeted Proteomics

NRicher™ Bead Platform Provides Unique Sub-Proteome Biases And Fit For Purpose Opportunities for Targeted LC-MS Quantification

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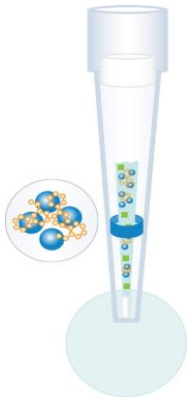
NRicher™ Beads Are Versatile to A Variety of Bead Processing Formats

In addition to standard spin-filter formats, other formats compatible with the 50 µm NRicher™ beads are:

High Throughput Automation Compatible INTip™ SPE (DPX Technologies) Format

Aspirate and dispense cycles mix NRicher™ beads and solutions



The INTip™ SPE tip format improves ease of use and scalability to process multiple samples in parallel, utilizing 96-well plates and automated liquid handlers. The tip-based formats have been proven to be compatible with most automation platforms, i.e., Integra, Hamilton, etc. Please inquire for more information, as these formats are customized to the application and automation platform.

96-Well Vacuum or Pressure Filter Format

The NRicher™ beads can be readily processed in 96-well filter formats. Please inquire.

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.**
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