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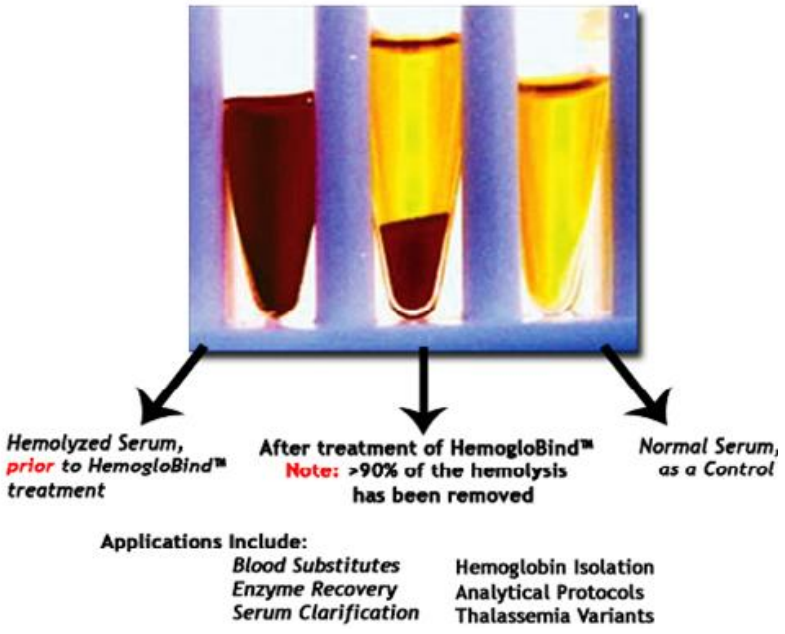
HemogloBind™

Hemoglobin Removal and Capture

- Has a high degree of specificity for hemoglobin, without cross-reacting with other proteins or analytes
- Suitable for
  - Hemolyzed serum/plasma
  - Whole Blood, Dried Blood Cards (DBS) or Erythrocyte lysates
  - Tissue Homogenates, compatible with RIPA buffer
- Applications in analytical interferences, enzyme monitoring, proteomics
- Species Agnostic
- Over 30 citations including analysis in cellular thermal shift assay (CETSA), LC-MS proteomics, Hemoglobin derivatives, Western blot, enzyme activity, ELISA
- Related product NuGel™HemogloBind™ comes in a dry powder format, compatible with high throughput 96-well automation, see https://www.biotechsupportgroup.com/NuGel-HemogloBind-Hemoglobin-Capture-Reagent-p/np-ho.htm

Poly-electrolytes are polymers with repeating units of stationary charges. **HemogloBind™** is derived from insoluble elastomeric poly-electrolytes that bind proteins through an empirically derived chemistry combining elements of polymer composition, and cross-linking architecture. As with bio-polymers like DNA and Heparin, governing their reactivity is the spatial presentation of electrostatic groups along a flexible polymer chain.

HemogloBind™ does not cross react with most common serum components, making it an excellent tool in numerous applications. These include analytical protocols where optical interference is problematic. Hemoglobin variants, as in thalassemia, bind with differential affinity towards **HemogloBind™**, though this has not been fully evaluated. For purification and/or analysis of hemoglobin, a modest elevation in pH will facilitate desorption from the polymer.



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Product	Size	Item No.
HemogloBind™	5ml	HO145-05
HemogloBind™	15ml	HO145-15
HemogloBind™	50ml	HO145-50

Specification

HemogloBind™ is supplied as an aqueous suspension of a synthetic polymer, pH 6.5. After centrifugation, the ratio of liquid to gel pellet is about 2 parts liquid, to 1 part pellet.

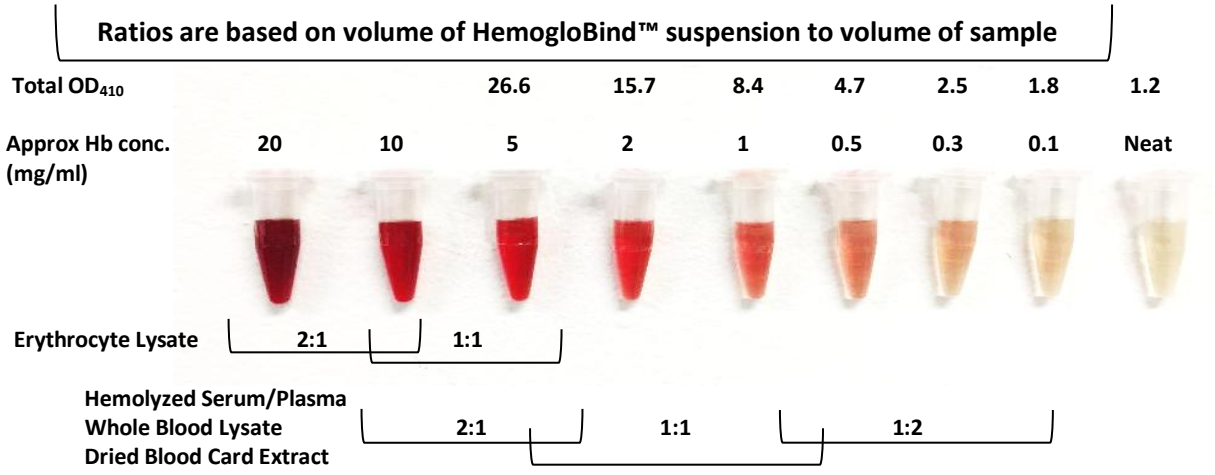
Storage

Supplied as an aqueous suspension of synthetic polymer, pH 6.5. The reagent when not used must be kept sealed and stored at 4°C. Do not freeze. HemogloBind™ retains full activity when stored at 4°C for 6 months. Expiration date is shown on label.

Notes: Hemoglobin concentrations in hemolyzed serum/plasma vary greatly. Protocols are intended as guidelines, but it is recommended to try different ratios of HemogloBind™ to sample for optimal results.

Spin-Filters (low protein binding, 0.45 µm filter element) are not supplied, but can be purchased separately, please inquire.

Guidelines for determining volume ratio of HemogloBind™ to sample. Use this chart to estimate the amount of Hemoglobin in samples containing mostly Hemoglobin (as in lysed erythrocytes) or mostly serum/plasma proteins (as in hemolyzed serum). Adjust volume ratio as necessary to optimize for investigative goals.



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PROTOCOL – To Treat 250 µl of Hemolyzed serum using microfuge tubes

1. Shake the HemogloBind™ suspension.
2. Using wide-bore pipette tips, pipette 250 µl or 500ul of the HemogloBind™ suspension.
3. Add 250 µl of the hemolyzed serum. (~10 mg Hb /mL)
4. Vortex for 30 seconds.
5. Mix for 10 minutes.
6. Centrifuge for 1-2 minutes at 9000 RPMs (8,000xg). Carefully aspirate the supernatant from the pellet.

Supernatant contains hemoglobin depleted sample suitable for further analysis, while the pellet contains the hemoglobin removed.

PROTOCOL – To Treat 250 µl of Hemolyzed serum in Spin-Filter Tube

1. Shake the HemogloBind™ suspension.
2. Using wide-bore pipette tips, pipette 250 µl or 500ul of the HemogloBind™ suspension into the filter of the Spin-Filter tube set.
3. Add 250 µl of the hemolyzed serum (~10 mg Hb/mL) to the HemogloBind™ suspension. Vortex for 20 seconds.
4. Mix for 10 minutes.
5. Centrifuge for 1-2 minutes at 9000 RPMs (8,000xg).

Filtrate contains hemoglobin depleted sample suitable for further analysis, while the retained material contains the hemoglobin removed.

PROTOCOL – To Treat Whole Blood Sample Using Microfuge Tube

1. Shake the HemogloBind™ suspension.
2. In a separate microfuge tube, to 10-20 µl of blood sample, add 100-200 µl 0.02M K_2HPO_4 pH 6.5. Vortex for 5 minutes.
3. Add 100-200 µl of HemogloBind™ suspension to the sample from step 2.
4. Vortex for 20 seconds, then mix well for 10 minutes at room temperature.
5. Centrifuge for 4 minutes at 9,000 rpm (8,000xg).
6. Collect the filtrate or supernatant which contains hemoglobin depleted sample, while the matrix contains the hemoglobin.

Supernatant contains hemoglobin depleted sample suitable for further analysis, while the pellet contains the hemoglobin removed.

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PROTOCOL – To Treat Whole Blood Sample Using Spin-Filter Tube

1. Shake the HemogloBind™ suspension.
2. Using the filter tube of the Spin-filter set, to 10-20 µl of blood sample, add 100-200 µl 0.02M K_2HPO_4 pH 6.5. Vortex for 5 minutes.
3. Using wide-bore pipette tips, pipette 100-200 µl of the HemogloBind™ suspension into the same sample Spin-Filter tube.
4. Vortex for 20 seconds. Mix for 10 minutes.
5. Centrifuge for 1-2 minutes at 9000 RPMs (8,000xg). Carefully aspirate the supernatant from the pellet.

Filtrate contains hemoglobin depleted sample suitable for further analysis, while the retained material contains the hemoglobin removed.

Desorption of Bound Hemoglobin

For purification and/or analysis of hemoglobin, 100 mM Tris-Borate, pH 9, will facilitate desorption of hemoglobin bound to HemogloBind™.

Hemoglobin Variants

Hemoglobin variants, as in thalassemia and glycosylated-hemoglobin, can also be analyzed with **HemoVoid™ - Hemoglobin Variant Enrichment**, see link https://www.biotechsupportgroup.com/HemoVoid-Hemoglobin-Variant-Enrichment-From-Blood-p/hbv.htm

Selection of HemogloBind ™ Reference Applications

Hemolyzed Serum Analyses

Neil Adrian P. Ondevilla, Peng-Wen Liu, Wan-Ting Huang, Tzu-Ping Weng, Nan-Yao Lee, Syu-Cing Ma, Jian-Jang Huang, Tak-Wah Wong, Hsien-Chang Chang, A point-of-care electrochemical biosensor for the rapid and sensitive detection of biomarkers in murine models with LPS-induced sepsis, Biosensors and Bioelectronics, Volume 254, 2024, 116202, ISSN 0956-5663, https://doi.org/10.1016/j.bios.2024.116202.

The article states "… Hemolysis is common in blood samples, which can interfere with the detection. Prior to the analysis, **the hemolyzed samples were pretreated for 15 min with HemogloBind™**…"

Red Cell Lysates

Chen, Yaozhen, et al. "Red blood cells undergo lytic programmed cell death involving NLRP3." *Cell* (2025).

The article states "**Hemoglobin was depleted by adding 250 µl of HemogloBind suspension** to each lysate. …the hemoglobin-depleted supernatant…was then subjected to liquid chromatography-tandem mass spectrometry (LC-MS/MS) analysis". Gene Ontology (GO) analysis revealed significant enrichment of pathways associated with cytoskeleton organization and actin filament assembly dynamics.

Sae-Lee, Wisath, et al. " The protein organization of a red blood cell ." *Cell Reports* 40.3 (2022): 111103.

"For hemolysate preparations, remnant white ghosts were removed by centrifugation at 21,000 ×g for 40 min at 4°C, and **the supernatant treated with HemogloBind (Biotech Support Group) in order to bind and remove free Hgb**."

Dziekan, Jerzy Michal, et al. "Cellular thermal shift assay for the identification of drug–target interactions in the Plasmodium falciparum proteome." Nature Protocols (2020): 1-41.

The cellular thermal shift assay (CETSA) enables proteome-wide target screening for unmodified compounds with undetermined mechanisms of action, providing quantitative evidence about direct drug–protein interactions. The workflow involves treatment of *P. falciparum*–infected erythrocytes with a compound of interest, heat exposure to

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denature proteins, soluble protein isolation, enzymatic digestion, peptide labeling with tandem mass tags, offline fractionation, and LC-MS analysis. The article states "**The intact-cell CETSA protocol features a HemogloBind-based sample processing step, which provides a relatively fast, reliable and inexpensive method to deplete >90% of hemoglobin from processed intact-cell samples. As a result, it leads to a 40-50% increase in the number of peptide spectrum matches (PSMs) …".**

Nguyen, Anthony T., et al. "UBE2O remodels the proteome during terminal erythroid differentiation." *Science* 357.6350 (2017): eaan0218. This study used multiplexed quantitative proteomics to identify candidate substrates of UBE2O, an E2 (ubiquitin-conjugating) enzyme, in an unbiased and global manner. **Because of the overly abundant presence of Hemoglobin, selective depletion of Hemoglobin was necessary.** The article states "Reticulocytes were lysed by vortexing for 5 minutes at room temperature… An additional 10 bed vol of **Hemoglobind™ suspension was added to the samples**, …. The supernatants, which contain hemoglobin-depleted sample, were … processed for TMT quantification.".

Whole Blood Lysates
de Boni, Laura, et al. "Aggregation-resistant alpha-synuclein tetramers are reduced in the blood of Parkinson's patients." *EMBO Molecular Medicine* (2024): 1-18. Synucleinopathies such as Parkinson's disease (PD) are defined by the accumulation and aggregation of the α-synuclein protein in neurons, glia and other tissues. In this study, an in vitro-cross-linking protocol for human EDTA-whole blood was used to determine the relative levels of disordered and higher-ordered multimeric forms of cytosolic α-synuclein in blood. The protocol incorporated **HemogloBind™ to remove interference from Hemoglobin**.

Hemoglobin Isolation and Derivative Analysis
Dasauni, Pushpanjali, et al. "Optimization and Identification of Single Mutation in Hemoglobin Variants with 2, 2, 2 Trifluoroethanol Modified Digestion Method and Nano– LC Coupled MALDI MS/MS." *Molecules* 27.19 (2022): 6357.
"The use of **Hemoglobind reduced the time to obtain pure Hb in an easy single–step procedure**. Pure Hb protein is required specifically to optimize and standardize methods for diagnostics. … The desorbed Hb was compatible with LC–MS, and other proteomics studies, as we verified, did not show any change in its intact mass either. **This one–step affinity purification gave us the utmost purified Hb**."

Tissue Lysates, LC-MS Proteomics
Jara, Zaira Palomino, et al. "Distinct Mechanisms of β-Arrestin–Biased Agonist and Blocker of AT1R in Preventing Aortic Aneurysm and Associated Mortality." *Hypertension* 80.2 (2023): 385-402.
The article states "Cleaned abdominal aortas were homogenized in T-PER Tissue Protein Extraction reagent…. **Abdominal aneurysm samples were treated to remove hemoglobin captured within the vessel wall. NuGel-HemogloBind … was used** according to the manufacturer protocol."

Species Agnostic – Applications to Different Species
Igoh, Akihisa, Masanobu Miura, and Satoru Miyaishi. "Animal Species and Blood Identification with Peptide Mass Fingerprinting." Analytical Chemistry (2024). https://doi.org/10.1021/acs.analchem.3c04314
The article states "We have developed the application of commercially available reagents for simply purifying hemoglobin from a wide range of animal species. …Blood, saliva, urine, semen, and sweat (20 μL); filter paper from which sweat was collected; and 0.5, 1, 2, and 5 μL of **pooled blood samples were treated with HemogloBind™** (BIOTECH SUPPORT GROUP, NJ, USA) to obtain hemoglobin solutions following manufacturer's protocol. …Hemoglobin was then dissociated according to the protocol to obtain a purified solution…."

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

CONTACT US

We welcome your questions, comments and concerns regarding our products.
Call 732-274-2866, Monday – Friday 9am-5pm EST.
Email sales@biotechsupportgroup.com