BIOTECH SUPPORT GROUP

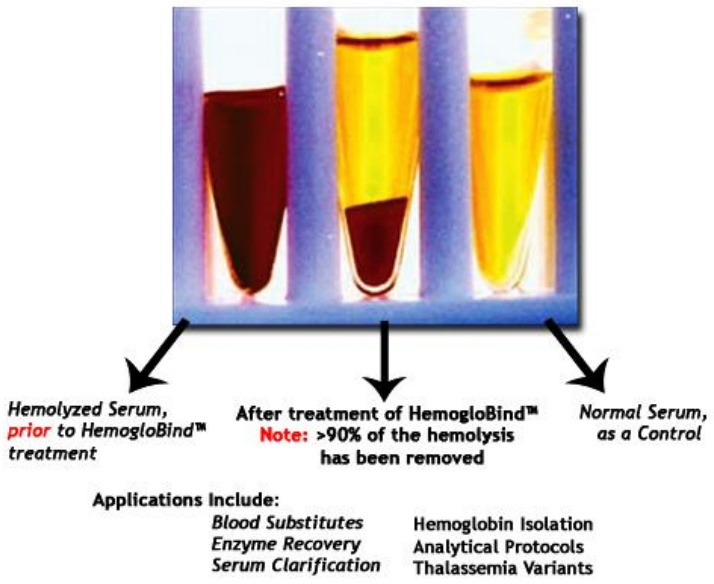
HemogloBind™

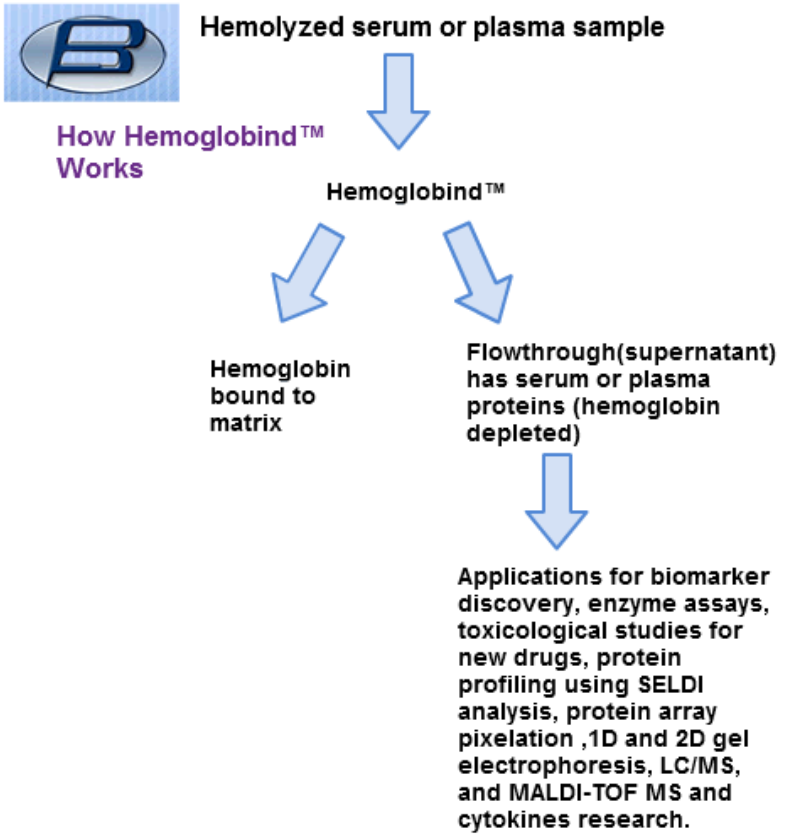
Hemoglobin Removal and Capture

- Has a high degree of specificity for hemoglobin, without cross-reacting with other analytes
- Suitable for
  - Hemolyzed serum/plasma
  - Whole Blood or Red Cell lysates
  - Tissue Homogenates
- Applications in analytical interferences, enzyme monitoring, blood substitutes

Poly-electrolytes are polymers with repeating units of stationary charges. HemogloBind™ comes from a class of solid-phase, or surface-based, elastomeric poly-electrolytic surfaces that bind proteins through an empirically derived chemistry combining elements of polymer composition, cross-linking architecture and charge properties. As with bio-polymers like DNA and Heparin, governing their reactivity is the spatial presentation of the 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, such as bilirubin analysis and bulk serum clarification. Hemoglobin variants, as in thalassemia, bind with differential affinity towards HemogloBind™. For purification and/or analysis of hemoglobin, a modest elevation in pH will facilitate desorption of hemoglobin bound to HemogloBind™.





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 2 parts liquid, to 1 part solid.

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 packing slip.

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PROTOCOL – To Treat 250 µl of Hemolyzed serum in SPIN-X 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-X set.
3. Add 250 µl of the hemolyzed serum to the small tub. (~10 mg/mL Hb)
4. Vortex for 20 seconds.
5. Mix by inversion for 10 minutes.
6. Centrifuge for 1-2 minutes at 9000 RPMs.

Filtrate contains hemoglobin depleted sample, while the solid contains the hemoglobin removed.

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/mL Hb)
4. Vortex for 30 seconds.
5. Mix by inversion for 10 minutes.
6. Centrifuge for 1-2 minutes at 9000 RPMs.

Supernatant contains hemoglobin depleted sample, while the solid contains the hemoglobin removed.

PROTOCOL – To Treat 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 Potassium Phosphate pH 6.5. Vortex for 5 minutes.
3. Add 100-200 µl of HemogloBind™ suspension to the sample from step 2.
4. Vortex or mix well for 10 minutes at room temperature followed by centrifugation for 4 minutes at 10,000 rpm.
5. Collect the filtrate or supernatant which contains hemoglobin depleted sample, while the matrix contains the hemoglobin.

Supernatant contains hemoglobin depleted sample, while the solid contains the hemoglobin removed.

PROTOCOL – To Treat Blood Sample Using SPIN-X Tube

1. Shake the HemogloBind™ suspension.
2. Using the filter of the SPIN-X set, to 10-20 µl of blood sample, add 100-200 µl 0.02M Potassium Phosphate pH 6.5. Vortex for 5 minutes.

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3. Using wide-bore pipette tips, pipette 100-200 µl of the HemogloBind™ suspension into the same sample SPIN-X filter.
4. Vortex for 20 seconds.
5. Mix by inversion for 10 minutes.
6. Centrifuge for 1-2 minutes at 9000 RPMs.

Filtrate contains hemoglobin depleted sample, while the solid contains the hemoglobin removed.

Other Analytes
HemogloBind™ cannot reduce interference caused by substances released from erythrocyte hemolysis other than that caused by hemoglobin. It is compatible with Bilirubin, Total Protein, Immunoglobulin, Albumin, Creatinine, ALT, AST, GGT, Creatine Kinase, LDH, BUN, Amylase, Cholinesterase. It is not compatible with Calcium, Magnesium. Suspension buffer contains trace amounts of Potassium Phosphate, which can be removed, please inquire.

Haptoglobin (HAP) Influence
The extent of hemoglobin removal may be influenced by the presence of elevated haptoglobin concentrations and sialo-glycoprotein which may be present in some acute-phase adult serum samples.

Myoglobin Binding
HemogloBind will not bind to Myoglobin, a protein that is structurally similar to hemoglobin but of lower molecular mass.

Hemoglobin Variants
Hemoglobin variants, as in thalassemia, and glycosylated hemoglobin bind with differential affinity towards HemogloBind™. This has not been fully characterized.

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™.

Featured HemogloBind ™ Applications –

Nguyen, Anthony T., et al. "UBE2O remodels the proteome during terminal erythroid differentiation." *Science* 357.6350 (2017): eaan0218.

During reticulocyte maturation, the proteome is remodeled through the programmed elimination of most generic constituents of the cell, in parallel with abundant synthesis of hemoglobin. The 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, which were then vortexed for another 10 min at room

temperature followed by 4 min of centrifugation at 10000 x *g*. The supernatants, which contain hemoglobin-depleted sample, were … processed for TMT quantification.”.

Hemolyzed Serum Exosome Analyses

Nishida-Aoki, Nao, et al. "Disruption of Circulating Extracellular Vesicles as a Novel Therapeutic Strategy against Cancer Metastasis." *Molecular Therapy* 25.1 (2017): 181-191. http://dx.doi.org/10.1016/j.ymthe.2016.10.009

This study considers that therapeutic strategies targeting cancer-derived extracellular vesicles (EVs) hold great promise because of the possibility they reposition microenvironments to accommodate metastasis. The researchers report on a novel strategy of therapeutic antibody treatment to target cancer-derived EVs and inhibit the metastasis of breast cancer in a mouse model. The article states “Hemoglobin was accumulated with HemogloBind™ beads (Biotech support group, Monmouth Junction NJ, USA) followed by 0.22 µm filtration. Then, the EVs in the sera were concentrated by ultracentrifugation…”.

Macromolecular Complexes and Functional Integrity

C Wan, B Borgeson, S Phanse, F Tu, K Drew, G Clark, et al. Panorama of ancient metazoan macromolecular complexes. Nature Volume:525, Pages:339–344 Date published:(17 September 2015). doi:10.1038/nature14877

HemogloBind™, contributed to this rigorous examination of protein complexes. When our products were used as a pretreatment step in the overall workflow, about twice the number of observations and annotations became possible. Furthermore, this study demonstrated the importance of a key feature implicit to all of our products; that is the maintenance of functional and structural integrity after separations. Without that particular feature, these additional observations would not have been possible.

Whole Blood
Lahut, Suna, et al. "Blood RNA biomarkers in prodromal PARK4 and REM sleep behavior disorder show role of complexin-1 loss for risk of Parkinson's disease." *Disease Models & Mechanisms* (2017): dmm-028035. http://dmm.biologists.org/lookup/doi/10.1242/dmm.028035

In this study, Parkinson’s disease progression is investigated through the accumulation and aggregation of the lipid-binding SNARE complex component alpha-synuclein (SNCA) which underlies vulnerability and defines its stages. The authors studied blood samples from a new large pedigree with *SNCA* gene duplication (PARK4 mutation), to identify effects of SNCA gain-of-function as potential disease biomarkers. The article states “For protein extraction from the EDTA tubes, 300 µl blood were lysed with equal amount of 1% SDS-RIPA buffer [50 mM Tris-HCl (pH 8.0), 150 mM NaCl, 1 mM EDTA, 1 mM EGTA, 1% Igepal CA-630 (Sigma), 0.5% sodium deoxycholate, 0.1% SDS, 1 mM PMSF and one tablet Complete Protease Inhibitor Cocktail (Roche)] and sonicated for 10 sec. The blood lysates were rotated at 4 °C for 30 min and centrifuged at 4 °C for 30 min. The supernatants were depleted in hemoglobin content using a commercial kit (HemogloBind™) following the manufacturer’s instructions”.

Chalásová, Katarína, et al. "Transketolase Activity but not Thiamine Membrane Transport Change in Response to Hyperglycaemia and Kidney Dysfunction." *Experimental and Clinical Endocrinology & Diabetes* (2017). https://www.thieme-connect.com/products/ejournals/abstract/10.1055/s-0043-115009

Diabetic kidney disease, a common complication of both type 1 and type 2 diabetes, is associated with significant morbidity and mortality, and represents the most common cause of chronic kidney disease. The study hypothesized that protective pentose phosphate pathway action in diabetes might be compromised by limited intracellular availability of an active transketolase cofactor thiamine diphosphate (TDP). To evaluate the levels of thiamine tranporter proteins in whole blood, t he article states " For protein isolation, whole blood aliquots were lysed with water and haemoglobin was removed using HemogloBind™ (Biotech Support Group) according to manufacturer's instructions…".

Species Agnostic – Applications to Different Species

Snider, Thomas H., Christina M. Wilhelm, Michael C. Babin, Gennady E. Platoff Jr, and David T. Yeung. "Assessing the therapeutic efficacy of oxime therapies against percutaneous organophosphorus pesticide and nerve agent challenges in the Hartley guinea pig."The Journal of Toxicological Sciences 40, no. 6 (2015): 759-775.

Acetylcholine is an essential neurotransmitter, and inhibitors of cholinesterases(ChEs) are potent toxins. A primary component of anti-organophosphorus therapy is an oxime reactivator to rescue inhibited acetylcholinesterases. For this, clinical signs of toxicity can be measured from blood cholinesterase [Acetylcholinesterase (AChE) and butyrylcholinesterase (BChE)] activity utilizing a modified Ellman's method. Biotech Support Group's unique solid-phase polymer for hemoglobin depletion, was used for pretreatment. The article states "Briefly, whole blood samples were treated with HemogloBind™ which interferes with the ChE activity assay due to spectral overlap."

Craig, J. R., et al. "A comparison of the anatomical and gastrointestinal functional development between gilt and sow progeny around birth and weaning." *Journal of animal science* (2019).

Gilt progeny (GP) often have restricted growth performance and health status in comparison to sow progeny (SP) from birth. To better understand underlying mechanisms, the study aimed to compare differences in growth and development between GP and SP in the first 24 h after birth and in the peri-weaning period. Because serum samples were quite hemolysed after collection and processing, it became necessary to use HemogloBind™ to allow for better detection of IgG by ELISA. The article states "As per the manufacturer's instructions, 250 µL of Hemoglobind was added to 250 µL of hemolyzed serum…

Parvathi S. Kumar, Haree K. Pallera, Pamela S. Hair, Magdielis Gregory Rivera, Tushar A. Shah, Alice L. Werner,Frank A. Lattanzio, Kenji M. Cunnion, and Neel K. Krishna. Peptide inhibitor of complement C1 modulates acute intravascular hemolysis of mismatched red blood cells in rats.TRANSFUSION Volume 00, May 2016. doi:10.1111/trf.13674.

In brief, the study evaluated the role of a peptide inhibitor of complement C1 (PIC1) in an animal model of acute intravascular hemolysis in both prevention and rescue scenarios. The authors state "To remove free Hb that may cause optical interference in bilirubin analysis, we treated all the samples with Hb depletion from hemolyzed serum/plasma (HemogloBind, Biotech Support Group). Bilirubin concentration was then measured with a Bilirubin Assay Kit (Sigma-Aldrich, St. Louis, MO)."

Other References

Blood Substitutes

Laing, Richard W., et al. "The use of an acellular oxygen carrier in a human liver model of normothermic machine perfusion." *Transplantation* 101.11 (2017): 2746.

Red cell lysates

O'Connell, Grant C., et al. "Monocyte-lymphocyte cross-communication via soluble CD163 directly links innate immune system activation and adaptive immune system suppression following ischemic stroke." *Scientific reports* 7.1 (2017): 12940

Kyoungsook Park, Christopher D. Saudek, and Gerald W. Hart Increased Expression of β-N-Acetylglucosamindase (O-GlcNAcase) in Erythrocytes from Prediabetic and Diabetic Individuals.Diabetes.2010;59(7):1845-50.

Delobel J., Rubin O., Prudent M., Crettaz D., Tissot J.-D., Lion N.(2010) Biomarker Analysis of Stored Blood Products: Emphasis on Pre-Analytical Issues. International Journal of Molecular Sciences. 11(11):4601-4617

Alvarez-Llamas, Gloria, Fernando de la Cuesta, Maria G. Barderas, Irene Zubiri, Maria Posada-Ayala, and Fernando Vivanco. "Characterization of Membrane and Cytosolic Proteins of Erythrocytes." In Vascular Proteomics, pp. 71-80. Humana Press, 2013.

Alvarez-Llamas, G., de la Cuesta, F., Barderas, M. G., Darde, V. M., Zubiri, I., Caramelo, C., Vivanco, F. A novel methodology for the analysis of membrane and cytosolic sub-proteomes of erythrocytes by 2-DE.Electrophoresis.2009;30:4095-4108

Zihao Wang, Kyoungsook Park, Frank Comer1, Linda C. Hsieh-Wilson, Christopher D. Saudek, Gerald W. Hart. Site-Specific GlcNAcylation of Human Erythrocyte Proteins: Potential Biomarker(s) for Diabetes Mellitus. Diabetes.2008;58, 309-317.

Yuichi Miki, Tomoki Tazawa, Kazuya Hirano, Hideki Matsushima, Shoko Kumamoto, Naotaka Hamasaki, Tomohiro Yamaguchi, Masatoshi Beppu. Clearance of oxidized erythrocytes by macrophages: Involvement of caspases in the generation of clearance signal at band 3 glycoprotein. Biochemical and Biophysical Research Communications.2007; 363(1):57-62

Sarawathi,et al., Relative quantification of glycated Cu-Zn superoxide dismutase in erythrocytes by electrospray ionization mass spectrometry, Biochimica et Biophysica Acta. 1999.1426(3):483-90

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