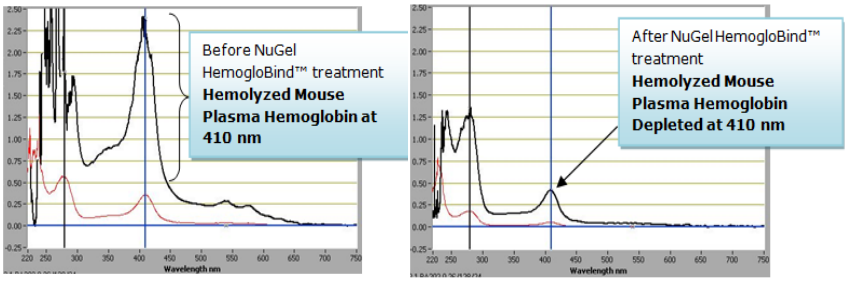
BIOTECH SUPPORT GROUP

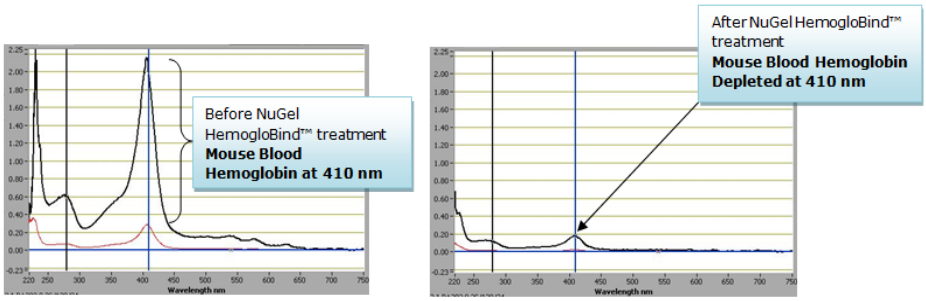
# NuGel-HemogloBind™

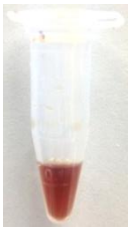
## *Hemoglobin Capture Reagent From Blood and Hemolyzed Serum with NuGel™ Matrix*

- Has a high degree of specificity for hemoglobin binding up to 10 mg/ml
- Removes hemoglobin from any species including human, sheep, bovine, goat, etc
- Removes hemoglobin from organs, tissues.
- Hemoglobin removal from red blood cell lysate for proteomics and biomarker drug discovery
- The flow through fractions(hemoglobin depleted) retain their enzymatic and biological activity
- The flow through fractions(hemoglobin depleted) is compatible with LC-MS, activity based protein profiling and proteomic studies.

NuGel-Hemoglobind™ is reengineered for increased stability. It is based on NuGel silica (50 microns in size, 1000Å) covalently bound to elastomeric polyelectrolytes. It binds >95% of hemoglobin from blood.



Hemolyzed Mouse Plasma before Treatment with NuGel

**NuGel HemogloBind™**

Hemolyzed Mouse Plasma after Treatment with NuGel HemogloBind

Diluted Hemolyzed Mouse Plasma Before Treatment

NuGel HemogloBind™

Diluted Hemolyzed Mouse Plasma After Treatment

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| Product | Size | Total Sample Processed | Item Price |
|---|---|---|---|
| **NuGel-HemogloBind™** | 25 preps | 500µl of blood or 5 ml of Hemolyzed Serum or Plasma | NP-HO-T25 |
| **NuGel-HemogloBind™** | 50 preps | 1 ml of blood or 10 ml of Hemolyzed Serum or Plasma | NP-HO-T50 |
| | | | |

| Items Required | 25 Prep | 50 Prep | Reagent |
|---|---|---|---|
| **NuGel-HemogloBind™** | 1.25 grams | 2.5 gram | Supplied |
| **Hemoglobin Binding Buffer (HB)** | 15 ml | 30 ml | Supplied |
| **SpinX Centrifuge tube filters** | 25 | 50 | Supplied |

## PROTOCOL – To Treat Blood Sample Using Microfuge Tube

1. Weigh out 50 mg of **NuGel-HemogloBind™** matrix in a microfuge tube.
2. Add 200 µl of **Hemoglobin Binding Buffer (HB)** to the matrix. Vortex or mix well for 2 minutes at room temperature.
3. In a separate microfuge tube, add 200 µl of **Hemoglobin Binding Buffer (HB)** and 10-20 µl of blood sample. Vortex for 3 minutes.
4. Add sample from step 3 to sample from step 2.
5. Vortex or mix well for 10 minutes at room temperature followed by centrifugation for 4 minutes at 10,000 rpm.
6. Collect the supernatant which contains hemoglobin depleted sample, while the matrix contains the hemoglobin.

## PROTOCOL – To Treat Hemolyzed Plasma or Serum Sample Using Microfuge Tube

1. Weigh out 50 mg of **NuGel-HemogloBind™** in microfuge tube and add 400 µl Hemoglobin Binding Buffer. Vortex for 2 minute.
2. Add 200 µl Hemolyzed Sample to step 1.
3. Vortex or mix well for 10 minutes at room temperature followed by centrifugation for 4 minutes at 10,000 rpm
4. Collect the supernatant which contains hemoglobin depleted sample, while the matrix contains the hemoglobin.

## PROTOCOL – To Treat Blood Sample Using Spin-X Tube

1. Weigh out 50 mg of **NuGel-HemogloBind™** matrix in a spin-tube.
2. Add 200 µl of Hemoglobin Binding Buffer. Vortex or mix well for 2 minutes at room temperature, centrifuge for 2 minutes at 10,000 rpm.
3. Discard the supernatant.

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4. In a separate microfuge tube, add 400 µl of Hemoglobin Binding Buffer to the 10-20 µl of blood sample. Vortex or shake for 3 minutes.
5. Add sample from step 4 to the pellet sample from step 3.
6. Vortex or mix well for 10 minutes at room temperature, and then centrifuge for 4 minutes at 10,000 rpm.
7. Collect the filtrate which contains hemoglobin depleted sample, while the matrix contains the hemoglobin.

## PROTOCOL – To Treat Hemolyzed Plasma or Serum Sample Using Spin-X Tube

1. Weigh out 50 mg of **NuGel-HemogloBind™** matrix in a spin-tube and add 200 µl Hemoglobin Binding Buffer. Vortex or mix well for 2 minutes at room temperature, centrifuge for 2 minutes at 10,000 rpm.
2. Discard supernatant.
3. In a separate microfuge tube, add 400 µl Hemoglobin Binding Buffer to 200 µl Hemolyzed sample. Vortex for 3 minutes.
4. Add the sample from step 3 to the pellet from step 2. Vortex or mix well for 10 minutes at room temperature followed by centrifugation for 4 minutes at 10,000 rpm
5. Collect the filtrate which contains hemoglobin depleted sample, while the matrix contains the hemoglobin.

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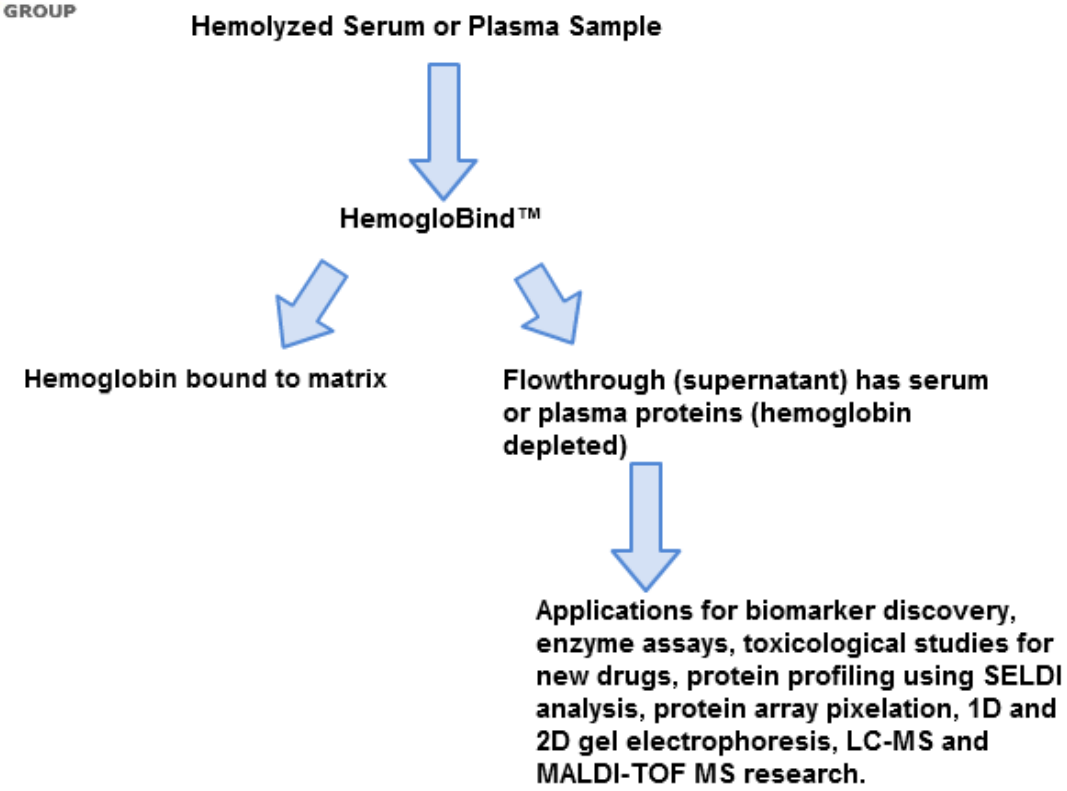
## How NuGel-HemogloBind™ Works




**Featured HemogloBind ™ Reference 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.".

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**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


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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

## CONTACT US

We welcome your questions, comments and concerns regarding our products.

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