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HemogloBind™ Blood Card Kit

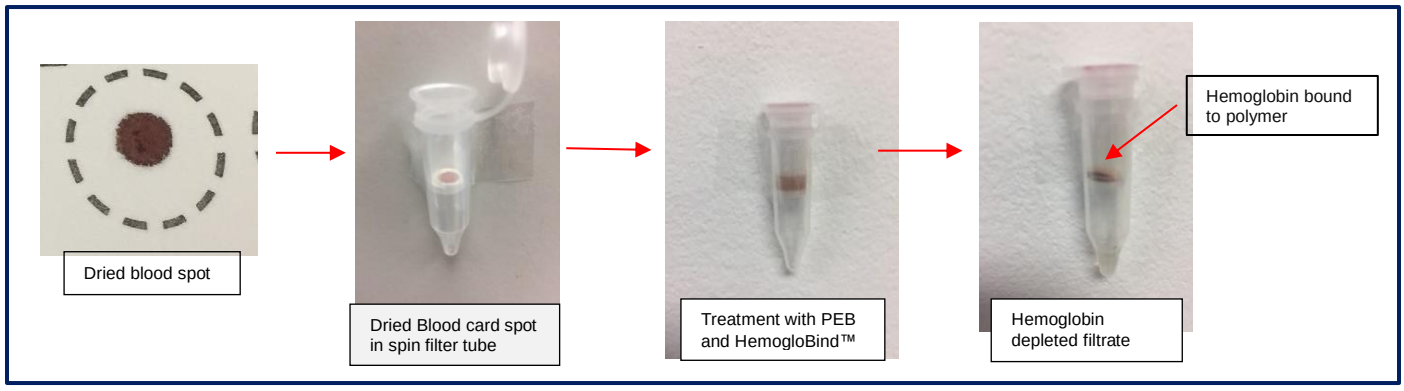
Hemoglobin Depletion and Protein Enrichment From Dried Blood Spots

- Dried blood spots are useful for low volume analyses, and simple collection and transport
- Protocols suitable for inexpensive whole blood card systems, no need for cell separation
- Hemoglobin binding >90%, with 30-45 minute spin-filter format
- Protocols based on ≤10 µl whole blood applied, but suspension format is flexible to most volumes
- Blood proteins and enzymes are enriched for biomarker and proteomic investigations.
- Removes hemoglobin from diverse species including human, sheep, bovine, goat, rat, mouse, etc.
- High throughput easily scalable.
- 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

Hemoglobin is a common contaminant from dried whole blood cards and not normally found in serum samples. The **HemogloBind™ Blood Card** protocol was designed to substantially reduce the presence of hemoglobin and its associated interference with many serum proteins and other analytes.

HemogloBind™ is engineered for a high degree of selectivity and does not cross react with most common serum components, making it an excellent sample prep in numerous applications. These include analytical protocols where optical interference is problematic, such as bilirubin and cholinesterase analysis.

Flow chart of HemogloBind™ Blood Card Application:



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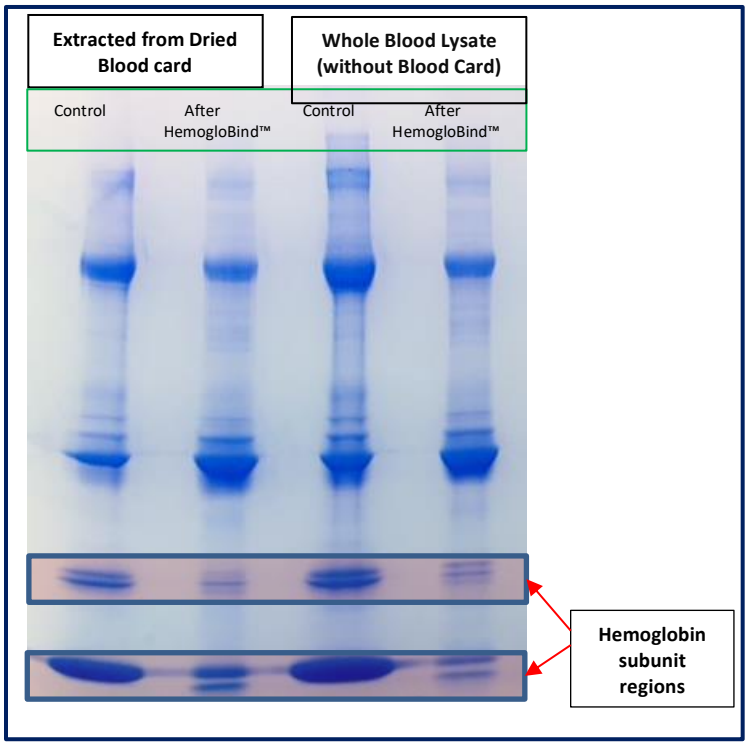
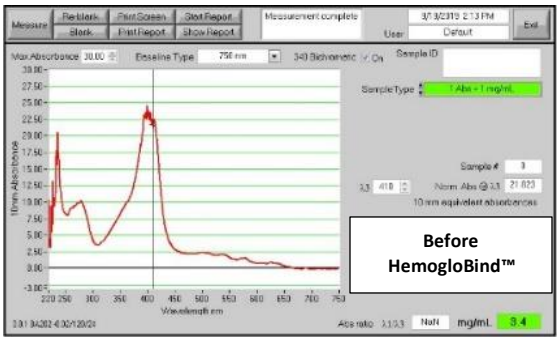
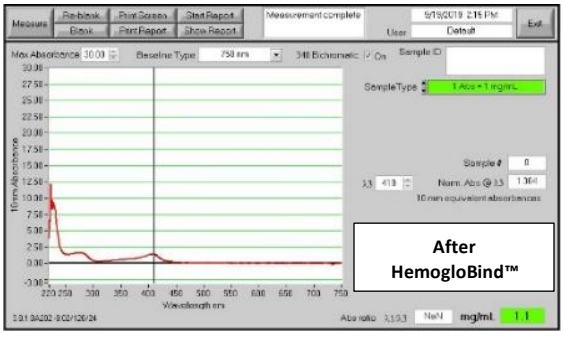
SDS PAGE showing comparison of hemoglobin removal from blood card kit and whole blood lysate



Absorbance at 410nm shows presence of hemoglobin.





Hemoglobin depletion and Protein Recovery from Dried Blood Spots

Dried Blood Spot (µl)	Hemoglobin Present (Based on 200mg Hb per ml blood) (mg)	Extraction Buffer (µl)	HemogloBind™ Reagent Used (µl)	Protein Recovery (µg)	Hemoglobin removal (%)
20	4	200	200	400 – 500	➢ 95%
10	2	100	100	200 – 250	➢ 95%
5	1	50	50	100 – 125	➢ 95%
2.5	0.5	25	25	90 - 100	➢ 95%

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Product	# of samples processed	Item No.
HemogloBind™ Blood Card	10 Dried Whole Blood Spot (7mm hole punch)	H0145BC-10
HemogloBind™ Blood Card	50 Dried Whole Blood Spot (7mm hole punch)	H0145BC-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.

Kit Content	10 Prep	50 Prep	Reagent
HemogloBind™	1 ml	5 ml	**Supplied**
Protein Extraction Buffer (PEB) (0.01M K Phosphate Dibasic, pH 7.0 adjusted with HCl)	1 ml	5 ml	**Supplied**
Spin Filter Tubes	10	50	**Supplied**
Suggested Or Equivalent Supplier of Blood Card: Whatman 903™ Protein Saver cards			**Not Supplied**

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

HemogloBind™ Protocol For Hemoglobin Depletion From Blood Spot/Blood Card

Based on processing ≤10 µl whole blood applied to and dried on Whatman 903™ Protein Saver cards (approximately equivalent to the 7mm circle)

Notes:
The centrifugation time may vary, adjust as necessary to get complete filtration.

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

1. **EXTRACTION OF DRIED PROTEIN FROM THE CARD.** Punch out the dried blood section from the card into a spin-filter assembly. Add 100 µl PEB buffer. Shake for 30 – 45 minutes at room temperature.

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2. **HEMOGLOBIN REMOVAL.** Shake the Hemoglobind™ suspension well before use. Using wide-bore (or cut) pipette tips, add 100 µl HemogloBind™ to the Spin-filter from step 1. Vortex or mix thoroughly for 10 mins and centrifuge at 10,000 rpm (8,000xg) for 5 mins.

The filtrate contains the hemoglobin depleted sample suitable for further analysis.

Selection of HemogloBind ™ Reference Applications

Hemolyzed Serum Analyses
Krishna, Neel K., and Kenji Cunnion. "Derivative Peptide Compounds and Methods of Use." U.S. Patent Application No. 15/192,934. http://www.freepatentsonline.com/y2016/0376322.html

The patent application describes synthetic peptide compounds for therapy and diagnostics of complement-mediated diseases, such as inflammatory diseases, autoimmune diseases, and microbial and bacterial infections and non-complement-mediated diseases, such cystic fibrosis and various acute diseases. The invention describes Peptide Inhibitors of Complement C1. In the example description, the patent states "Due to large amounts of hemolysis in the latter time points and the associated optical interference in bilirubin analysis, all the samples were pre-treated with HemogloBind™ (Biotech Support Group, NJ) prior to analysis with the Bilirubin Assay Kit."

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
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…EVs in the sera were concentrated by ultracentrifugation…".

Red Cell Lysates
Hojo-Souza NS, de Azevedo PO, de Castro JT, Teixeira-Carvalho A, Lieberman J, et al. (2020) Contributions of IFN-γ and granulysin to the clearance of Plasmodium yoelii blood stage. PLOS Pathogens 16(9): e1008840. https://doi.org/10.1371/journal.ppat.1008840

The authors investigated how Plasmodium infection induces MHC-I expression on Retics. In addition, whether granulysin helps control Plasmodium infection *in vivo* has not been studied. To remove interferences associated with Hemoglobin, the article states, "For western blot analysis, erythroblasts pellets were resuspended in RIPA Buffer (Sigma)…. The Retics were treated with HemogloBind …".

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) protocol presents a comprehensive strategy for the identification of drug targets. CETSA enables proteome-wide target screening for unmodified antimalarial compounds with undetermined mechanisms of action, providing quantitative evidence about direct drug–protein interactions. The experimental workflow involves treatment of *P. falciparum*–infected erythrocytes with a compound of interest, heat exposure to denature proteins, soluble protein isolation, enzymatic digestion, peptide labeling with tandem mass tags, offline fractionation, and liquid chromatography–tandem mass spectrometry (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) for *P. falciparum* and non-hemoglobin human proteins.".**

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, 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.".

Whole Blood Lysates
Kaneko, Tomonori, et al. " System-wide hematopoietic and immune signaling aberrations in COVID-19 revealed by deep proteome and phosphoproteome analysis. " Research Square preprint (2021).

The author's goals were to gain systems-level insights into SARS-CoV-2 pathogenesis. For that, they compared the blood proteome and phosphoproteome of ICU patients with or without SARS-CoV-2 infection, and healthy control subjects by quantitative mass spectrometry. To remove the highly abundant amount of Hemoglobin, the article states "Hemoglobin was depleted from PBMC whole cell lysate samples according to HemogloBind ™ manufacturer instruction with modifications."

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

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 transporter 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…".

Hemoglobin Isolation and Derivative Analysis
Yamagishi, Yoshikazu, Hirotaro Iwase, and Yasumitsu Ogra. "Post-Mortem Changes of Methomyl in Blood with Hemoglobin." Chemical Research in Toxicology.

In this study, the researchers considered specific methomyl hemoglobin adducts detected by liquid chromatography quadrupole time-of-flight mass spectrometry (LC-Q/TOF-MS). To help isolate Hemoglobin, the article states "Hb was separated with HemogloBind in accordance with the manufacturer's instructions." The authors conclude that one Hemoglobin derivative, the W-adduct could be used as a biomarker of methomyl poisoning.

Tissue Lysates, LC-MS Proteomics
Heather E. McKiernan, Phillip B. Danielson, Catherine O. Brown, Masha Signaevsky, Christian G. Westring and Kevin M. Legg, Developmental Validation of a Multiplex Proteomic Assay for the Identification of Forensically Relevant Biological Fluids, Forensic Science International, (2021) https://www.sciencedirect.com/science/article/pii/S0379073821002280?via%3Dihub

The aim of this study was to validate a multiplex proteomic assay for the identification of target peptide fragments by multiple reaction monitoring on a triple quadrupole mass spectrometer originating from tissue-specific proteins. The article states "If samples contained excessive quantities of hemolyzed red blood cells, four volumes of HemogloBind™ were added." The authors conclude that the mass spectrometry-based workflow offers significant advantages compared to existing serological methods.

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 (**HemogloBind™ & NRicher™ 6**) were used as a pretreatment step in the overall workflow, 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.

Species Agnostic – Applications to Different Species

D'Alessandro, Angelo, et al. "Hematologic and systemic metabolic alterations due to Mediterranean type II G6PD deficiency in a novel murine model." *bioRxiv* (2021).

To generate a more accurate model of Glucose 6 phosphate dehydrogenase deficiency, the human sequence for a severe form of G6PD deficiency (Med -) was knocked into the murine G6PD locus and confirmed by Western blot. The article states "Briefly, RBCs were washed 3 times PBS, followed by transfer of one part washed RBCs into three parts water, followed by end over end rotation for 5 min at room temperature to lyse the RBCs. Lysed RBCs were then mixed 1:1 with HemogloBind™, followed by end over end rotation for 10 min at room temperature. HemogloBind™ and bound hemoglobin were pelleted by centrifugation, and supernatants subjected to an additional hemoglobin depletion with HemogloBind™. Supernatants were used for western blotting."

Southwell, Rebecca Marie, Kenneth Sherlock, and Matthew Baylis. "Cross-sectional study of British wild deer for evidence of Schmallenberg virus infection." Veterinary Record (2020).

The purpose of this study was to survey wild deer across Great Britain for recent evidence of Schmallenberg virus (SBV). Postmortem blood samples were tested for SBV antibodies. Because of the presence of Hemoglobin interference in many samples, the article states "**In order to avoid poor quality samples yielding false ELISA results, 59 samples estimated to have above 50mg/dL and less than 250mg/dL haemoglobin concentration, according to their colour, were selected for treatment with HemogloBind™** (Biotech Support Group, New Jersey, USA).".

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.

Clinical signs of cholinesterase inhibitor 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). Bilirubin concentration was then measured with a Bilirubin Assay Kit (Sigma-Aldrich, St. Louis, MO)."

Blood Substitutes

Juriasingani, Smriti, et al. "Evaluating the Effects of Subnormothermic Perfusion with AP39 in a Novel Blood-Free Model of Ex Vivo Kidney Preservation and Reperfusion." *International Journal of Molecular Sciences* 22.13 (2021): 7180.

This study evaluated whether adding hydrogen sulfide donor AP39 to Hemopure, a blood substitute, during subnormothermic perfusion improves kidney outcomes. Because of the added Hemopure, the article states, "Most of the urine samples collected were heavily pigmented, due to the presence of hemoglobin from the Hemopure, which prevented the use of conventional urinalysis

methods. A 1:3 dilution of urine in Hemoglobind (Biotech Support Group, Monmouth Junction, NJ, USA) allowed us to obtain clearer urine samples after 10 min of vigorous shaking and centrifugation at 12,000× *g*."

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.

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

RELATED SAMPLE PREP PRODUCTS:

Albumin & IgG Removal products:
https://www.biotechsupportgroup.com/Albumin-Removal-s/307.htm

Lipid Removal Reagent and Clarification products:
https://www.biotechsupportgroup.com/Lipid-Removal-s/316.htm

CONTACT US

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

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