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HemogloBind™ Blood Card Kit

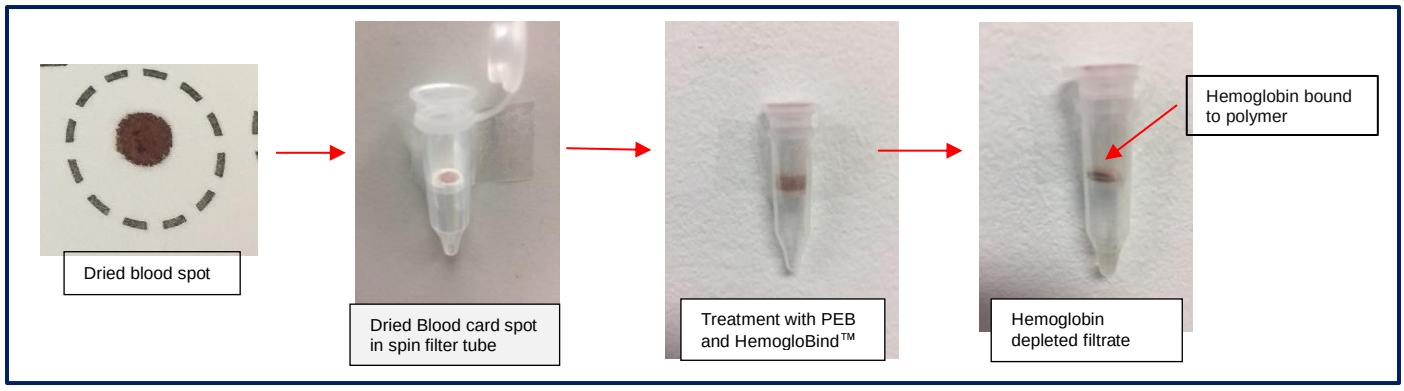
Hemoglobin Depletion and Protein Enrichment From Dried Blood Spots

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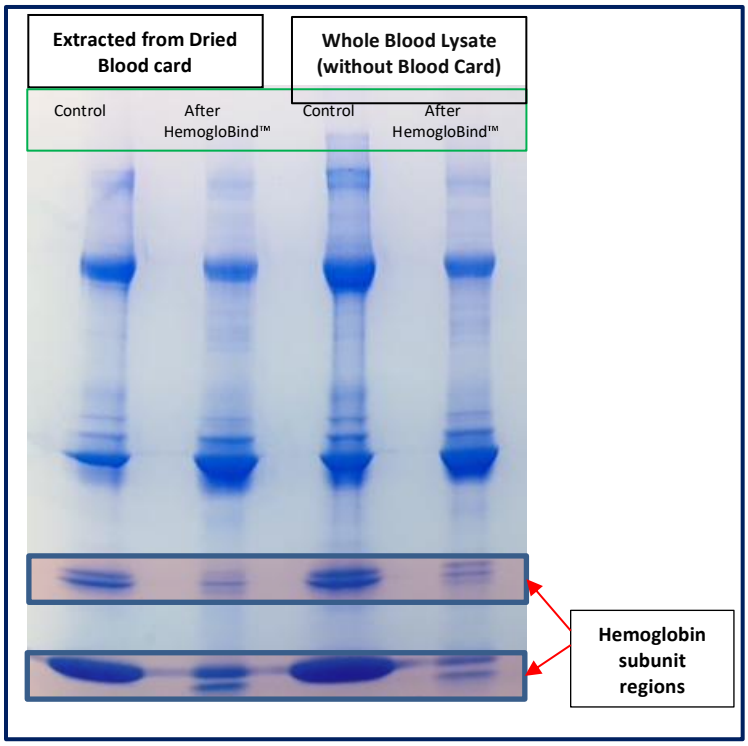
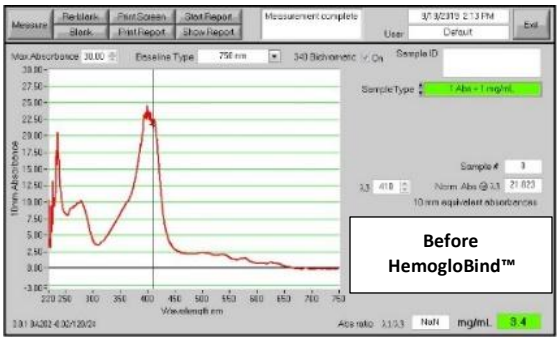
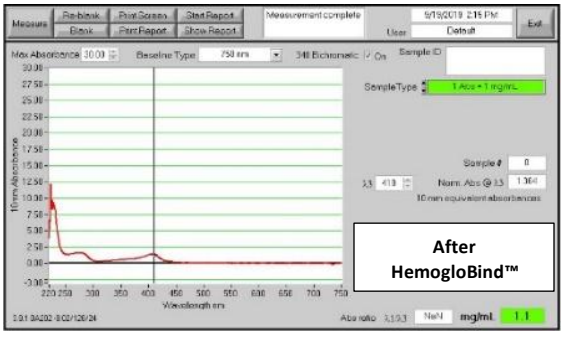
SDS PAGE showing comparison of hemoglobin removal from blood card kit and whole blood lysate



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.

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. Centrifuge for 1-2 minutes at 9000 RPMs (8,000xg). Carefully aspirate the supernatant from the pellet. The filtrate contains the hemoglobin depleted sample suitable for further analysis.

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**."

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**."

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**