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HemogloBind™ Blood Card Kit

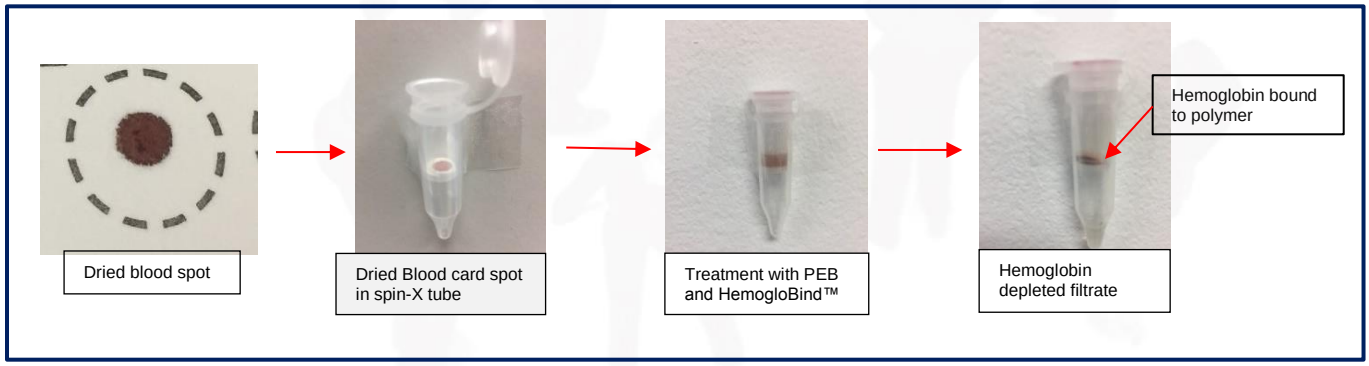
Hemoglobin Depletion and Protein Enrichment From Dried Blood Spots

- 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.

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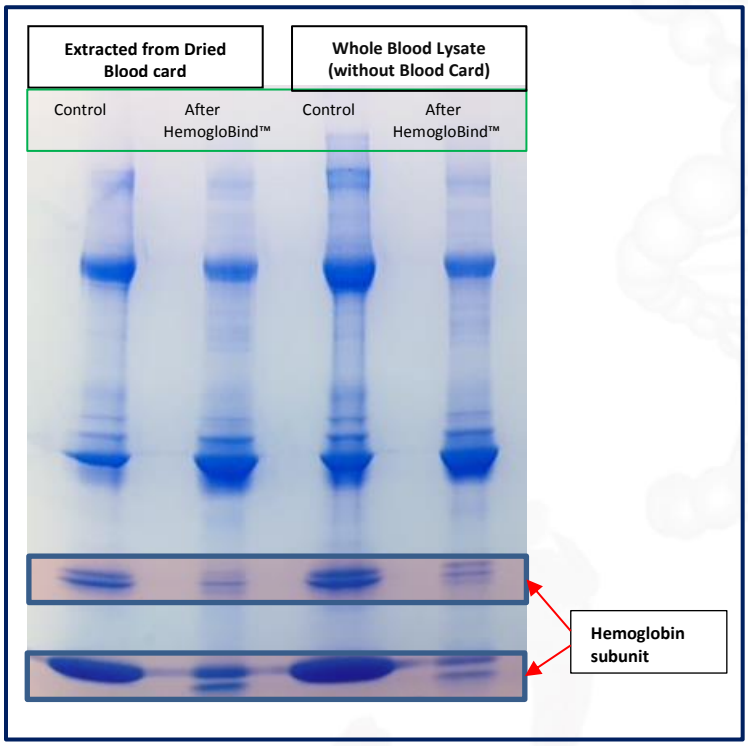
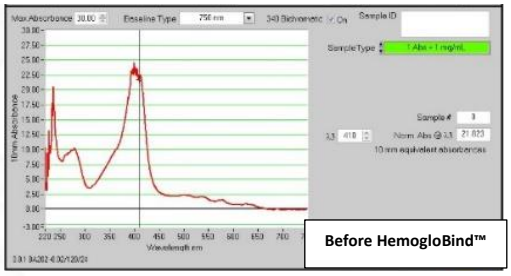
SDS PAGE showing comparison of hemoglobin removal from blood card kit and whole blood lysate

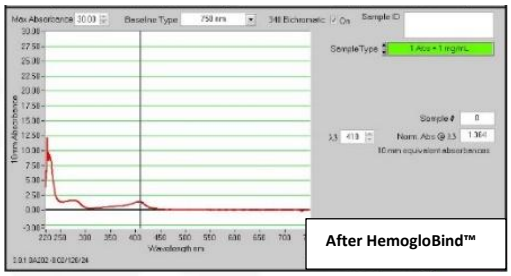


Absorbance at 410nm shows presence of hemoglobin.

Graph showing high concentration of hemoglobin before HemogloBind™



Graph showing reduced hemoglobin concentration after HemogoBind™



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 (ml)	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

Kit Content	10 Prep	50 Prep	Reagent
HemogloBind™	1 ml	5 ml	**Supplied**
Protein Extraction Buffer (PEB)	1 ml	5 ml	**Supplied**
Spin-X Centrifuge tube filters	10	50	**Supplied**
Suggested Or Equivalent Supplier of Blood Card: Whatman 903™ Protein Saver cards			**Not Supplied**

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)

1. Extraction of dried protein from the card: Punch out the dried blood section from the card into a spin-X tube. Add 100 µl PEB buffer. Shake for 30 – 45 minutes at room temperature .
2. For Hemoglobin Removal: Shake the Hemoglobind™ suspension well before use. Using wide-bore (or cut) pipette tips, add 100 µl HemogloBind™ to the Spin-X tube from step 1. Vortex at 1,800 rpm for 10 mins and centrifuge at 10,000 rpm (or highest microfuge speed) for 5 mins.
3. The filtrate contains the hemoglobin depleted sample suitable for further analysis.

HemogloBind™ is supplied as an aqueous suspension of a synthetic polymer, pH 6.5. The reagent should 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.
The PEB buffer supplied is 0.01M potassium phosphate Dibasic, pH 7.0 adjusted with HCl.

HemogloBind™ References

Dried Blood Spots Citations:

Michelle R. Robinson, Lei Guo; Raymond J. Gonzalez; Kara M. Pearson; Kevin P. Bateman; Daniel S. Spellman. Differentiating Modes of Drug Induced Liver Injury Using Parallel Reaction Monitoring LC-MS. ASMS Conference 2017 poster report. "Hemoglobin depletion improves PRM panel coverage in DBS and volumetric adsorptive microsampling (VAMS)".

Hakuna, Lovemore, et al. "A simple assay for glutathione in whole blood." Analyst (2015). (http://pubs.rsc.org/en/content/articlelanding/2015/an/c5an00345h) "…Hb can be removed using a commercial product, HemogloBind™, which can isolate and remove up to 90% of blood Hb."

Other HemogloBind™ Citations:

Red cell lysates
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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).

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

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)

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Stored Blood Products
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Whole Blood.
McGarry, Kevin G., et al. Evaluation of HemogloBind™ treatment for preparation of samples for cholinesterase analysis. (2013). Advances in Bioscience and Biotechnology, 2013, 4, 1020-1023

Red blood cells
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Hikosaka, Keisuke, et al. "Deficiency of Nicotinamide Mononucleotide Adenylyltransferase 3 (Nmnat3) Causes Hemolytic Anemia by Altering the Glycolytic Flow in Mature Erythrocyte"*Journal of Biological Chemistry*(2014): jbc-M114.

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CONTACT US

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