BIOTECH SUPPORT GROUP
Sample Prep that Matters

HEMOVOID™ LC-MS ON-BEAD DIGEST REFERENCE APPLICATION

Introduction

For years the protein depletion toolkit was limited primarily to immuno-affinity chromatography and other biologically-derived tools. While effective for many applications, such tools are not efficient for "omics" sample preparation, as throughput, economy and simplicity are especially required. Furthermore, these same separation tools often denatured proteins which limited there use in applications which demanded the measurement of function, structure or bio-activity. For these reasons, BSG has been dedicated to create new methods and applications to drive efficient workflows and better data quality for all proteomic and biomarker analyses.

To achieve these goals, BSG has developed a chemical library of general non-specific adsorbents, or stated another way - beads with weak affinity or imperfect fit interactions. Without the use of antibodies, progressive displacement allows the beads to bias for or against certain proteins, without compromising protein integrity.

Three BSG products support Hemoglobin Removal applications:

- **HemogloBind™** & **NuGel™ HemogloBind™** for selective binding of Hemoglobin &
- **HemoVoid™** for negative selection or voidance of Hemoglobin with consequent enrichment of the remaining erythrocyte proteome on the bead

NuGel™ products were empirically characterized to meet the needs of the application; for example, **HemoVoid™** to selectively void (not bind) Hemoglobin with special binding bias towards the vast majority of the remaining low abundance erythrocyte proteome to the bead. **HemogloBind™** is different; it is a suspension product derived from a family of acid-alcohol elastomeric co-polymers. These polymers are synthesized to have separation characteristics like salts and solvents, but with the mechanical advantages of solid-phases: simple removal of the bound macromolecules with no solute carryover, and adaptability to filtration, centrifugation, and automation. **NuGel™ HemogloBind™** has similar separations performance but is supplied as a dry powder, rather than as a liquid suspension.

The BSG Advantage

All of our products have these 4 features in common:

1. *Consumable Use:* not derived from biologicals, no regeneration, cost-effective, no specialized instruments or HPLC.
2. *Functional Integrity:* retains enzymatic and biological activity for functional and chemical proteomics.
3. *Enrichment or Depletion:* strategies for both enrichment of low abundance proteomes, or depletion of high abundance proteins.
4. *On-Bead Digestion:* improves performance and workflow, unique proteolytic efficiencies.

This application report features the **BSG On-Bead Digestion Advantage using HemoVoid™**.

Personal Correspondence.

HemoVoid™ *On-Bead Digestion prior to LC-MS analyses.*

The following **HemoVoid™** data was provided by Irene Granlund, Umeå University, Umeå, Sweden. It shows a comparison of Trypsin digestion of the **HemoVoid™** bead-bound sub-proteome compared a more conventional solution digest. On left is the protocol using on-bead digestion method, where the proteins are reduced and digested while they remain bound to the bead; vs. on right, the filter-aided solution digestion where the proteins are first eluted from the bead and then digested using a membrane filter format.

Digestion on HemoVoid™ Matrix

Courtesy of Irene Granlund, Umeå University, Umeå, Sweden

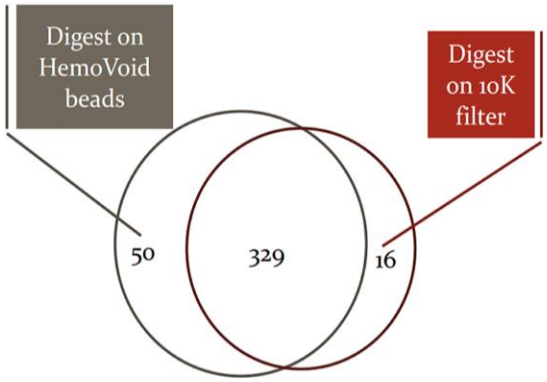
Digestion on Spin-X® tube

1. After the final wash steps from Protocol provided for HemoVoid™, add 100 µL of 5 mM DTT in HVWB to the beads for complete immersion, mix and incubate at 60°C for 1 hour.
2. Add 100 µL of 25 mM Iodoacetamide in HVWB (end concentration 12.5 mM) to the DTT/bead suspension, mix and incubate in the dark, 37°C for ½ hour.
3. Centrifuge at 3,200 x g for 2 minutes, and discard supernatant. Wash the beads with 200 µL HVWB, mix and centrifuge at 3,200 x g for 2 minutes.
4. Move the HemoVoid™ device with Hemoglobulin depleted proteins to new clean tubes.
5. Add 100 µL of digestion solution (5ng/µL trypsin in 50 mM NH_4HCO_3-solution) to the beads. Incubate 37°C, overnight (15 hours).
6. Centrifuge down at 3,200 x g for 2 minutes.
7. Wash HemoVoid™ matrix 2 times with 150 µL 50 mM NH_4HCO_3, mix and centrifuge down, at 3,200 x g for 2 minutes.

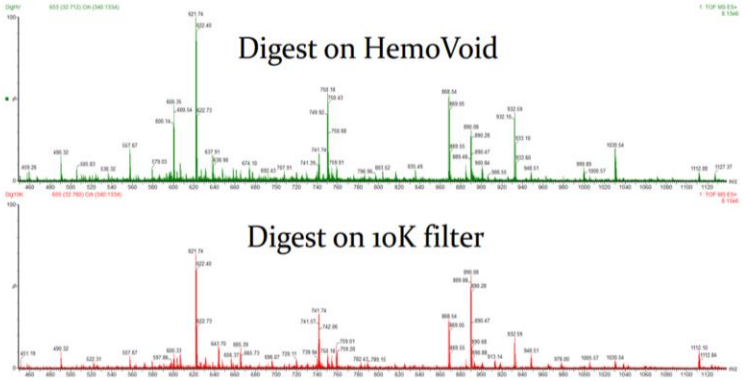
Digestion on 10K filter device

1. The proteins are eluted according to the Protocol provided with 300 µL Elution Buffer HVEB. Mixed for 10 minutes and centrifuged 4 min. at 9,000 x g.
2. The eluted sample is transferred to 10K filter device, (NanoSep 10K Omega, centrifugal device, Pall Life Sciences). The samples are centrifuged down on the filter (the solution under filter is thrown away).
3. Add 300 µL 19.5 mM DTT in Guanidine solution (6 M guanidine, 0.1 M Tris, 5 mM EDTA, pH ~8). Incubate at 60°C for 1 hour.
4. Add 100 µL 81 mM Iodoacetamide in Guanidine solution (end concentration 20 mM). Alkylate in the dark, 37°C for ½ hour.
5. Spin down 14000 x g to remove liquid.
6. Wash filter two times with 200 µL 50 mM NH_4HCO_3, spin down at 14 000 x g in between.
7. Move the 10K filter to new clean tubes.
8. Add 100 µL of digestion solution (5ng/µL trypsin in 50 mM NH_4HCO_3-solution) on the filter. Incubate 37°C, overnight (15 hours).
9. Wash filter with 2 times with 150 µL 50 mM NH_4HCO_3. Spin down at 14 000 x g.

IDENTIFIED PROTEINS



SPECTRUM



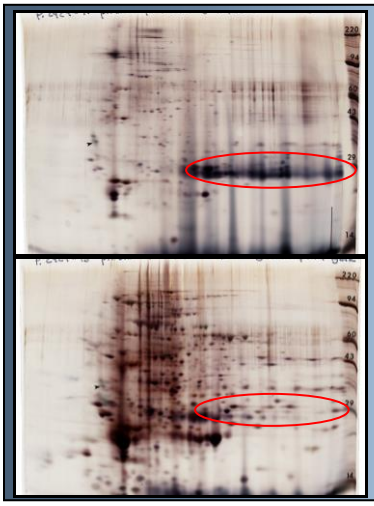
Hemoglobin Removal Kits

- Unique surface chemistries, no antibodies
- depletes Hemoglobin 90-95%

HemoVoid™

Hemoglobin Depletion For Erythrocyte Proteomics

- Hemoglobin voids in flow-through, applicable to red cells, heavily hemolyzed serum, whole blood and dried blood spot (DBS) card
- Low abundance protein and enzyme enrichment
- Consumable, cost-effective
- Mild elution maintains native structure with retained enzymatic, functional and bio-activities
- Compatible with LC-MS, activity-probe profiling and virtually all proteomic analyses

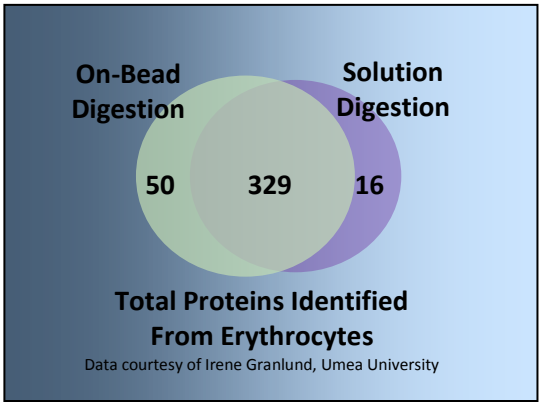


2DE Comparison. Red circles indicate the Hemoglobin subunits region. The HemoVoid™ eluate (bottom) has been severely depleted of Hemoglobin. The remainder of the red cell proteins are substantially enriched (visualized) and are better resolved in the HemoVoid™ eluate. Many more proteins are detectable after HemoVoid™ treatment with extensive proteome coverage across both dimensions.

HemoVoid™ LC-MS On-Bead

Hemoglobin depletion plus low abundance protein enrichment with optimized on-bead digestion for LC-MS erythrocyte & whole blood proteomics

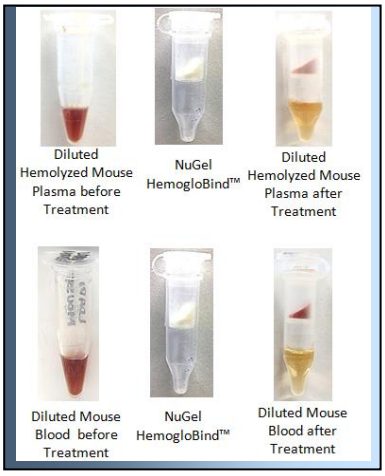
- Seamless workflows,
- Unique proteolytic efficiencies
- Label, label free & phospho- compatible



HemogloBind™ & NuGel™ HemogloBind™

Removes Hemoglobin Interference

- Highly specific for hemoglobin binding
- depletion from hemolyzed serum, dura, BALF, and whole blood
- Functional integrity maintained with simple transfer to post-treatment interrogations
- supports biomarker tests

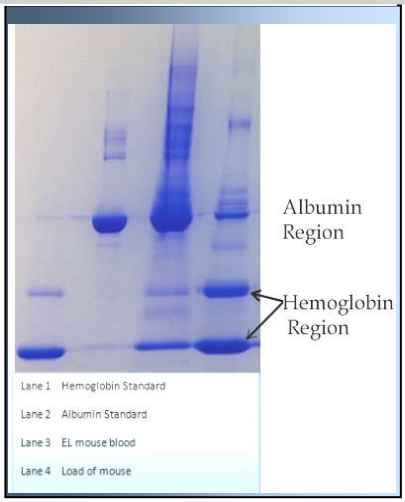


Hemoglobin Removal Trial Kits

Product	Includes
HemoTrial™ Kit	5 ml HemogloBind™ + 5 Preps NuGel™ HemogloBind™ + 5 Preps HemoVoid™
HemogloBind™ Trial Kit	5 ml HemogloBind™ + 5 Preps NuGel™ HemogloBind™

HemoVoid™ Blood Card Kit

The HemoVoid™ Blood Card kit substantially reduces hemoglobin interference from dried blood spot protein analytes



HemoVoid™ Hemoglobin Variant Enrichment from Blood

Purification & Enrichment Of Hemoglobin From Blood For Hemoglobin Variant Research

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