Proteomic Sample Prep & Enrichment

BIOTECH SUPPORT GROUP

Sample Prep that Matters

Albumin & IgG Removal Kits
- Unique surface chemistries
- Depletes 90-95%
- Species agnostic

https://www.biotechsupportgroup.com/Articles.asp?ID=451

Hemoglobin Removal Kits
- Unique surface chemistries
- Depletes 90-95%
- Species agnostic

https://www.biotechsupportgroup.com/Articles.asp?ID=452

Lipid Removal & Clarification
- Extensively cited
- Replaces hazardous hydrocarbons
- Diverse samples

https://www.biotechsupportgroup.com/Articles.asp?ID=456

Sample Prep – Mass Spectrometry
- Proteome Enrichment
- Not based on immuno-affinity
- Bead assisted sample prep (BASP™)

https://www.biotechsupportgroup.com/Articles.asp?ID=432

Sample Prep - Proteomic Liquid Biopsy
- Urine
- Synovial
- Saliva/Sputum
- Blood
- Broncheolavage
- Exosome

https://www.biotechsupportgroup.com/Articles.asp?ID=457

Class Specific Enrichment
- Glycoproteins
- Virus
- Kinases

https://www.biotechsupportgroup.com/Articles.asp?ID=453

The BSG Advantage

4 common features and collective advantages:

Consumable
Cost-effective, not derived from biologicals

Enrichment / Depletion
Diverse strategies, species agnostic

Functional Integrity
Maintained throughout separations

On-Bead Digestion
Efficient workflows, quality LC-MS/MS data

www.biotechsupportgroup.com

BSG's Collective Advantages as Represented in Journal References

Consumable

Species Agnostic

Phillippi, Danielle T., et al. "Inhaled diesel exhaust particles result in microbiome-related systemic inflammation and altered cardiovascular disease biomarkers in C57Bl/6 male mice." *Particle and fibre toxicology* 19.1 (2022): 1-29.
Exposure to particulate matter (PM) is a known mediator of inflammation and CVD. However, the role of inhaled traffic-generated PM on the gut microbiome and its corresponding systemic effects are not well-characterized. Thus, the study investigated exposure to inhaled diesel exhaust particles (DEP) on mice. To determine systemic inflammation, **following HemogloBind™ treatment, plasma concentrations of 20 Cytokines** were measured using a **Mouse** Cytokine/Chemokine Magnetic Bead Panel; the article stating "HemogloBind was added to plasma samples (1:1 ratio), …

Consumable

Not derived from immuno-affinity

Vialaret, Jerome & Kadi, Sarah & Tiers, Laurent & O Flynn, Robin & Lehmann, Sylvain & Hirtz, Christophe. (2018). Albumin depletion of human serum to improve quantitative clinical proteomics. Current Topics in Peptide & Protein Research 19. 53-62.
http://www.researchtrends.net/tia/abstract.asp?in=0&vn=19&tid=26&aid=6192&pub=2018&type=3
The article states "In comparison, methods using antibodies needed at least one-half day more. The albumin depletion method allowed to save precious time. ". The authors concluded that the **AlbuVoid™ depletion method proved to be faster and more cost-effective than antibody based methods**, and could be helpful for biomarker enrichment and detection in medical research.

On-Bead Digestion

Liu, Wenli, et al. "Erythroid lineage Jak2 V617F expression promotes atherosclerosis through erythrophagocytosis and macrophage ferroptosis." *The Journal of Clinical Investigation* (2022).
To explore underlying defects promoting oxidative changes in Jak2VF Red Blood Cells (RBC), unbiased proteomics profiling was conducted. The article states "…**haemoglobin removal and on-bead digestion**, …was based on the protocol in **HemoVoid™** kit. … Samples were first digested with LysC in HVWB (from kit) with protease: protein ratio 1:100 overnight at 37 °C and then with trypsin in HVWB (from kit) with protease: protein ratio 1:30."

Functional Integrity

David L. Wang, Chuanguang Xiao, Guofeng Fu, Xing Wang and Liang Li. "Identification of potential serum biomarkers for breast cancer using a functional proteomics technology". Biomarker Research (2017) 5:11.
As a pre-treatement enrichment, AlbuVoid™ was shown to enrich enzyme activity for subsequent analysis. As a result, additional candidate fractions become available to choose from for further biological validation as potential biomarkers. The article states "The **most dramatic difference for enzyme activity detection in using the AlbuVoid™ for serum protein enrichment was demonstrated.** … Compared with the direct serum proteinase measurement, both the levels and species of proteases were increased significantly in the enriched serum sample. …protease activity in the direct serum analysis suggested that the protease levels in the serum were below the detection threshold of protease activity…,**and it is necessary to use AlbuVoid™ to enrich these low level proteases to bring them to a high enough level to be detected**."

Enrichment/ Depletion

Nguyen, Anthony T., et al. "UBE2O remodels the proteome during terminal erythroid differentiation." *Science* 357.6350 (2017). The mechanisms that drive reticulocyte transition to red blood cell in terminally differentiating cells remain largely unclear. 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, …were … processed for TMT quantification**.".

Enrichment/ Depletion

Lange, Philipp F., et al. "Annotating N termini for the human proteome project: N termini and Nα-acetylation status differentiate stable cleaved protein species from degradation remnants in the human erythrocyte proteome." *Journal of proteome research* 13.4 (2014): 2028-2044.
The article describes a goal of the Chromosome-centric Human Proteome Project to identify all human protein species. With 3,844 proteins annotated as "missing" this is challenging. Enucleated and largely void of internal membranes and organelles, erythrocytes are simple yet proteomically challenging cells due to the high hemoglobin content (about 97% by mass) and wide dynamic range of protein concentrations that impedes protein identification. Using a N-terminomics procedure called TAILS, the authors identified 1369 human erythrocyte natural and neo-N-termini and 1234 proteins. From the **HemoVoid™ treated, hemoglobin-depleted soluble fraction, 778 proteins were identified, 171 of which were not represented in either the soluble non-depleted fraction or the membrane fraction**.

Consumable

Taylor SW, Clarke NJ, Chen Z, McPhaul MJ. A high-throughput mass spectrometry assay to simultaneously measure intact insulin and C-peptide. Clin Chim Acta. 2016 Apr 1;455:202-8. doi: 10.1016/j.cca.2016.01.019. The authors simultaneously measured intact endogenous-insulin and derived C-peptide, to help predict development of diabetes mellitus, as well as in differential diagnosis in cases of hypoglycemia. **Cleanascite™ is shown both to improve LC-MS measurements, and validated in accordance with CLIA '88 guidelines.**

Functional Integrity

Wang, Xueyu, et al. "Epigenetic Silencing of miR-33b Promotes Peritoneal Metastases of Ovarian Cancer by Modulating the TAK1/FASN/CPT1A/NF-κB Axis." *Cancers* 13.19 (2021): 4795. To examine whether fatty acids in OCM are the main source of energy for tumors, all fatty acids in OCM were first removed by **Cleanascite™** Lipid Removal Reagent. XTT cell viability analysis was performed and showed that the cell growth rate of ES-2 and MES-OV cells was remarkably reduced when cocultured in lipid-depleted OCM and "both miR-33b overexpression and **depletion of fatty acids by Cleanascite in OCM significantly impaired ovarian cancer cell migration and invasion**."