ALBUMIN REMOVAL REFERENCE APPLICATIONS

FEBRUARY 8, 2017

BIOTECH SUPPORT GROUP

Sample Prep that Matters

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 were not efficient for "omics" sample preparation, when throughput, economy and simplicity were required. Furthermore, these same separation tools often denatured proteins which limited there use in applications which required the measurement of function, structure or bio-activity. BSG is 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. For albumin depletion, choose from: **AlbuSorb™** for binding of albumin, **AlbuSorb™ PLUS** for combined albumin & immunoglobulin depletion, **AlbuVoid™** for negative selection or voidance of albumin with low abundance enrichment of remainder sub-proteome, and **AlbuVoid™ LC-MS On-Bead** for on-bead digestion of the enriched serum sub-proteome. All remove 90-95% albumin, regardless of species in simple spin-filter formats.

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.

The applications and references for use of these products follows.

Serum Biomarkers

Haiyan Zheng, Caifeng Zhao, Swapan Roy, Devjit Roy MD, Amenah Soherwardy, Ravish Amin, Matthew Kuruc. The Commonality of the Cancer Serum Proteome Phenotype as analyzed by LC-MS/MS, and Its Application to Monitor Dysregulated Wellness. Poster Reprint First Presented At AACR Annual Meeting 2016 Conference, Held April 17-20, 2016 New Orleans, LA USA.

> This research suggests there are biomarkers dysregulated regardless of the primary tumor, stage of progression or tumor burden. The study which collaborated with the Rutgers Center for Integrative Proteomics shows a most distinguishable pattern of early dysregulation observable as a cancer serum phenotype to date. New observations after using **AlbuVoid™ LC-MS On-bead** protocols include: Variant sub-populations (also known as proteoforms) of a common blood protein – Alpha-1-Antitrypsin, with functional reporting features severely distinguishable between cancer patients and normal/healthy individuals, and a measurable serum cancer profile that can be modeled with categorical biomarker proteins taken from inflammation, blood coagulation, tissue remodeling, and glycolysis, as well as new markers of unknown function.

Haiyan Zheng, Caifeng Zhao, Swapan Roy, Devjit Roy MD, Amenah Soherwardy, Ravish Amin, Matthew Kuruc. The Comparison of the Serum Proteome in Individuals with Cancers versus those without Cancer, and its application to Wellness Poster reprint first presented at 12th Annual US HUPO 2016 Conference, held March 13 – 16, 2016 Boston, MA, USA.

> Biomarker discovery from genomic analysis of singlular biomarkers or protein panels can be enhanced by doing albumin depletion experiments and on bead digestion. Using **AlbuVoid™ LC-MS On-bead** protocols, over 200 spectrally quantified proteins from healthy/normal or disease cancerous sera of pancreatic, breast, lung patients are obtained. Individuals from pancreatic, breast and lung cancer sera are analyzed by adding a binding buffer. The bead retains serum proteome and albumin is depleted. Subsequently the serum proteins on bead are analyzed by wash, reduction, alkylation, trypsin digestion and are labelled. Spectra from liquid chromatography mass spectrometry (LC-MS/MS) runs and proteins analyzed by Xtandem with protein filters are obtained. An example of a protein is the SERPIN family protease inhibitor. It allows validating quantitative proteomics data, discovering newly annotated serum proteome.

Swapan Roy, Matthew Kuruc. The Functional Subproteomes of Serpin Protease Inhibitors are Now Open for LC-MS Biomarker Discovery. MOJ Proteomics Bioinform 2016, 3(6): 00106

> The authors consider that the conformational variants of the unique family of protease inhibitors annotated as SERPINs, are most often underrepresented in proteomic analyses. This limits understanding the complex regulation that this family of proteins presents to the networks within the protease web of interactions. Using bead-based separation provided by the NuGel™ family of proteomic enrichment products - notably **AlbuVoid™** & **AlbuSorb™**, the authors demonstrate their utility to satisfy investigations of serum SERPINs. The authors also suggest their use to develop functional profiles of the SERPIN Proteoform, and how those can establish relationships to disease phenotypes, gene mutations, and deregulated mechanisms.

Zheng et al. AlbuVoid™ Coupled to On-Bead Digestion - Tackling the Challenges of Serum Proteomics . J Proteomics Bioinform 2015, 8:9.

> Serum samples can offer unique challenges in LC-MS proteomic analyses including: 1) the high abundance of Albumin accounting for about 50% of the total protein mass and, 2) proteolytic resistance, in large part due to substantial amount of glycosylation, a modification that manifests proteolytic resistance. In this short report, we describe new methods using a surface/bead based product, **AlbuVoid™**, which depletes Albumin through a negative selection or voidance strategy, retaining the vast amount of the remaining serum proteome on the bead. We then combine this novel enrichment, with a direct and seamless integration with Trypsin digestion, a method conventionally referred to as on-bead digestion. We evaluated the digestion time as a parameter to identify whether different sub-populations of peptides and proteins can be observed by LC-MS analyses. Using 2 different allotted digestion times - 4 hours, and overnight, each with a singular 3 hour gradient LC-MS run, between 400-500 total

proteins were observed for both human and rat sera, with overlapping and distinct sub-populations observable at each digest time. These results support that the described methods gain efficiencies over other high abundance depletion and in-solution digestion workflows. We solicit that such workflows will minimize many of the inconsistencies of proteolytic hydrolysis for both discovery and quantitative serum proteomic applications.

Application Report. AlbuSorb™ Product Extension combines Albumin and Immunoglobulin Depletion in a Consumable Format

From a foundational NuGel™ platform chemistry, a library of bead architectures has been created to support proteomic enrichment. These beads are general non-specific protein adsorbents, or stated another way - beads with weak affinity or imperfect fit interactions. Two of our products support Albumin Removal: **AlbuSorb™** for selective binding of Albumin & **AlbuVoid™** for negative selection or voidance of Albumin with consequent enrichment of the remaining serum proteome on the bead. We now report on adding Immunoglobulin depletion as an extension to **AlbuSorb™**. A LC-MS/MS analysis on human serum revealed between 500-600 total proteins, many of which are qualitatively and quantitatively biased to sub-proteomes either depleted of Immunoglobulins, or not depleted of Immunoglobulins. **AlbuSorb™ PLUS** designates and distinguishes **AlbuSorb™** without immunoglobulin depletion, from **AlbuSorb™** with Immunoglobulin depletion. Here we compare proteomic data derived from **AlbuSorb™**, **AlbuSorb™ PLUS** (which includes Immunoglobulin depletion), and **AlbuVoid™**.

Holmberg, Rebecka, Essam Refai, Anders Höög, Rosanne M. Crooke, Mark Graham, Gunilla Olivecrona, Per-Olof Berggren, and Lisa Juntti-Berggren. "Lowering apolipoprotein CIII delays onset of type 1 diabetes." *Proceedings of the National Academy of Sciences* 108, no. 26 (2011): 10685-10689

The glycoprotein Apolipoprotein C-III (apoCIII) inhibits lipolysis and its expression is documented to play a vital role in the development of hypertriglyceridemia when increased. The aim of this study was to determine apoCIII's increased levels in serum in T1D patients and that it affects the function and survival of pancreatic β cells in vitro. Authors Holmberg et al used **AlbuSorb™** to remove albumin from rat serum samples. Scientists setup experiments implementing the animal model diabetes-prone BB rat (DPBB) to ascertain if apoCIII increases contributed to calcium increase and B-cell death in vivo. To evaluate such levels, scientists used **AlbuSorb™** (Biotech Support Group) to remove albumin from serum samples. Scientists discovered that treating prediabetic animals with an antisense against apo CIII prolonged diabetes onset.

Tang MX, Ogawa K, Asamoto M. Effects of Nobiletin on PhIP-Induced Prostate and Colon Carcinogenesis in F344 Rats Nutrition and Cancer.2011;63(2):227-33

Authors showed how 0.05% citrus flavonoid nobiletin inhibited PhIP-induced rat prostate and colon carcinogenesis. **AlbuSorb™** was used for albumin depletion from serum samples to enable leptin expression. Following this, serum samples were diluted and denatured in the presence of sodium dodecyl sulfate and 2-mercaptoethanol by heating at 100◦C for 5 min. Then, proteins in each sample were electrophoretically. To prevent, nonspecific binding on the membranes 5% skim milk at room temperature for 1 h, followed by incubation with a polyclonal rabbit antileptin antibody allowed for leptin expression by western blot.

Holmberg, Rebecka Apolipoprotein CIII and Ljungan virus in diabetes 2010. Doctoral Thesis

Lowering the levels of apolipoprotein CIII is beneficial to prevent development of type 1 diabetes. **AlbuSorb™** was used on isolated islet cells from sera in prediabetic rats undergoing antisense treatment. **AlbuSorb™** removed >90% albumin from serum samples. The method involved adding serum to a binding buffer with **AlbuSorb™** powder followed by mixing and centrifugation. The supernatant was collected, freeze dried in 100ml 0.1% TFA and run on ACE C18 column 20-60%. The apolipoprotein CIII elutes were analyzed with by area under the curve measurements. Analysis of apolipoprotein CIII was done using MALDI mass spec.

Lu Q, Zheng X, McIntosh T Development of different analysis platforms with LC-MS for pharmacokinetic studies of protein drugs. Analytical Chemistry.2009;81(21):8715-23

> Using **AlbuSorb™**'s albumin depletion method first and then digest the depleted albumin solution (flow through fraction) for the subsequent LC-MS analysis of peptides, either 1-dimensional LC or 2-dimensional LC (ion exchange and reversed phase) with MS analysis. In this paper, authors use **AlbuSorb™** from Biotech Support Group in a sample of serum (i.e., 30 µL) containing the protein drug along with a binding buffer provided (i.e., 250 µL) and then 40 mg of **AlbuSorb™** powder is added in a spin-tube. At room temperature, the sample was mixed for 5-10 min on a rotating shaker, the spin-tube was centrifuged for 2 min, and the supernatant was collected for further analysis.

Serum Proteomics / On-Bead Digestion

Application Report: "AlbuVoid™ LC-MS On-Bead - Differential Expression of Lung & Breast Cancer Sera Proteins Using Quantitative (iTRAQ) Proteomics":

> Using the new **AlbuVoid™ LC-MS On-Bead** product, we spectrally quantified over 200 total proteins, 21 of which were differentially observed as either over or under expressed in the cancer sera. These results support new efficiencies for serum proteomics. We solicit that such workflows will minimize many of the inconsistencies of proteolytic hydrolysis for both discovery and quantitative serum biomarker applications.
> Download the application report from the Biotech Support Group website:
> http://biotechsupportgroup.com/sites/default/files/AlbuVoid™%20LC-MS%20On-Bead%20Differential%20Expression%20of%20Lung%20&%20Breast%20Cancer%20Sera%20Proteins%20Using%20Quantitative%20(iTRAQ)%20Proteomics%20Application%20Report.pdf

Serum Functional Proteomics

Sun, Zhenyu, Xiaofeng Chen, Gan Wang, Liang Li, Guofeng Fu, Matthew Kuruc, and Xing Wang. "Identification of functional metabolic biomarkers from lung cancer patient serum using PEP technology." Biomarker Research 4, no. 1 (2016): 1.

> In brief, the article's authors report a on a functional proteomics top-down method to systematically monitor metabolic enzyme activities in resolved serum proteins produced by a modified 2-D gel separation and subsequent Protein Elution Plate, a method collectively called PEP. The article states "Since most of the functional proteins or enzymes exist at relatively low level in the human serum and there is a limited loading capacity on the 2-DE gel, it is important to enrich the low abundance proteins before 2-DE and PEP analysis. **AlbuVoid™** (Biotech Support Group, Monmouth Junction NJ) has been shown to effectively enrich low abundance serum proteins while depleting the Albumin…whereby more functional features were observed with **AlbuVoid™** than without…". The study identified several potential functional enzyme biomarkers from lung cancer patient serum and evidence that the methods provide an alternative and complementary approach to sequence annotation for the discovery of biomarkers in human diseases.

Xing Wang, Michael Davies, Swapan Roy and Matthew Kuruc. Bead Based Proteome Enrichment Enhances Features of the Protein Elution Plate (PEP) for Functional Proteomic Profiling . Proteomes 2015, 3(4), 454-466 . doi: 10.3390/proteomes3040454

> In this short case study, the enrichment of select sub-populations of proteins is beneficial to systematically analyze protein functions of a whole enzyme family from an entire proteome. **AlbuVoid™** was used to remove Albumin and enrich the low abundance proteome, noting that distinguishable activity features are presented from the lung cancer vs. the normal sera. **KinaSorb™** was used to enrich for both a narrow spectrum substrate profile—Hexokinase activity, and a broad-spectrum protein kinase activity. The number of observable features was consistent with such narrow and broad-spectrum activities. **AlbuVoid™** enrichment and PEP processing proved suitable for profiling the functional activities of Hexokinase, Protease and Alkaline Phosphatase. These enzyme feature profiles are indicative of the functional diversity that can be generated, annotated and compared within and between sample phenotypes, using the combined methods.

Xing Wang, Ph.D., Zhenyu Sun, M.D., Xiaofeng Chen, M.D., Xiong Su, Ph.D., Gan Wang, Ph.D., Matthew Kuruc. Genetic Engineering News Jan 1, 2015 (Vol. 35, No. 1) OMICS Tutorial Discovery of Functional Serum Biomarkers, Exploring Cancer's Signature in the Sensitive Functional Domain of the Human Proteome.

A Genetic Engineering News article describes a joint collaboration with ArrayBridge (St. Louis, MO). The article describes the combination of first low abundance protein enrichment/high abundance protein depletion with the Biotech Support Group product – **AlbuVoid™**, followed by a modified 2-dimensional electrophoretic separation, and transfer via the ArrayBridge PEP plate into microplates. From there, the resolved functionally active proteins were measured and characterized.

Plasma Biomarkers

Espes, Daniel, Joey Lau, and Per-Ola Carlsson. "Increased circulating levels of betatrophin in individuals with long-standing type 1 diabetes." Diabetologia(2013): 1-4.

Authors Espes et al published an article in the journal Diabetologia which discovered an increase in hormone betatrophin in type 1 diabetics as compared to health individuals. Betatrophin causes an increase of pancreatic β cell replication and regulates glucose levels. Found in liver and adipose tissue, the hormone also increases β cell mass expansion. The article states: "Plasma samples were depleted of albumin using AlbuVoid Albumin Depletion Kit (Biotech Support Group, Monmouth Junction, NJ, USA). Data were normalized for total protein content." Betatrophin increases proliferation of beta cell and this study identified double the concentration of betatrophin as measured by western immunoblot using a betatrophin primary antibody in type 1 diabetics. Moreover factors such as age in healthy controls displayed a direct relationship with increase in betatrophin whereas triacylglycerol, LDL-cholesterol, HDL-cholesterol levels and insulin were not affected. Increasing concentrations of betatrophin did not prevent against the loss of C-peptide suggesting type 1 diabetes on betatropin treatment would benefit from combination treatment. The authors concluded, "An intervention in patients with type 1 diabetes with betatrophin treatment might require supraphysiological dosing as well as combination with immune regulatory treatment."

Urine

Zubiri, Irene, et al. "Diabetic nephropathy induces changes in the proteome of human urinary exosomes as revealed by label-free comparative analysis." *Journal of proteomics* 96 (2014): 92-102.

Proteinuria in urine samples causes contamination of urine samples for proteomics research. Sample preparation protocols with depletion could complement precipitation techniques as less contamination is present in exosomal fractions upon depletion, enrichment, concentration and sample clarification. The article cites protocols of proteomic workflows for biomarker discovery involving isolating exosomes from urine. Urine exosome isolation via ultracentrifugation upon performing depletion provides researchers with knowledge of renal regulation and could lead to the identification of biomarkers for diabetic nephropathy and diabetic mellitus. By removing albumin using **AlbuSorb™**, authors identified more urinary proteins such as flotillin-2, lamp-1, PODXL, tsg-101 from exosome fractions of urine samples. Upon depletion of high abundance proteins such as albumin, urine exosomes from diabetic and health controls were analyzed by LC-MS/MS and selected reaction monitoring (SRM). The research cites AMBP, MLL3, VDAC1 as proteins in urinary exosomes of diabetic nephropathy patients.

Cerebrospinal Fluid

Gwenael Pottiez, Pawel Ciborowski. Proteomic Profiling of Cerebrospinal Fluid Expression Profiling In Neuroscience. Neuromethods.2012;64:245-270

Authors Pottiez et al published a chapter in the book Expression Profiling in Neuroscience, Neuromethods titled, Proteomic Profiling of Cerebrospinal Fluid, on proteomic profiling platforms which analyze cerebrospinal fluid (CSF) for protein biomarkers and developing protein profiles of CSF for early identification of neurological diseases. Authors provide examples of affinity-based systems for removing most abundant proteins and cite **AlbuSorb™** albumin depletion kit from Biotech Support Group. Moreover variations of protein concentration yielded by immunodepletion of CSF samples from nondemented (ND) patients and patients with HIV-associated dementia (HAD) are recorded.

Synovial fluid

Happonen, K. E., Fürst, C. M., Saxne, T., Heinegård, D., & Blom, A. M. (2012). PRELP protein inhibits the formation of the complement membrane attack complex. Journal of Biological Chemistry, 287(11), 8092-8100.

> Approximately 65% of the total protein in normal synovial fluid consists of human serum albumin. Authors Kaisa E. Happonen et al cite **AlbuSorb™** for albumin depletion from synovial fluid for detecting and measuring Proline arginine rich end leucine-rich repeat protein (PRELP). Scientists demonstrated that PRELP inhibits MAC by decreasing C9 polymerization, thereby preventing limiting complement attack on basement membranes. Proper identification of low-abundance proteins in synovial fluid that may prevent disease biomarkers discovery is enhanced by albumin depletion. After using **AlbuSorb™**, synovial fluid proteins on two-dimensional polyacrylamide gel electrophoresis (2D-PAGE) are studied. The report demonstrates that Tandem mass spectrometry (MS/MS), coupled with multidimensional liquid chromatography (LC) and database searching is effective for protein identification and characterization. Examples of elevated biomarker candidates which are elevated in erosive or nonerosive rheumatoid arthritis (RA) are G3PDH,Peptidylprolyl isomerase, Cystatin B, Phosphoglycerate mutase 1, α2-plasmin inhibitor, S100A8 (calgranulin A), IgG1H Nie, Thymosin-β4.

Serum Containing Cell Culture Biomarkers

Narain, Niven Rajin, Rangaprasad Sarangarajan, Vivek K. Vishnudas, and Michael Andrew Kiebish. "USE OF MARKERS IN THE IDENTIFICATION OF CARDIOTOXIC AGENTS AND IN THE DIAGNOSIS AND MONITORING OF CARDIOMYOPATHY AND CARDIOVASCULAR DISEASE." U.S. Patent 20,140,100,128, issued April 10, 2014.

> Narain, Niven Rajin et al authored a patent on methods for detecting biomarkers of cardiovascular diseases, monitoring disease progression and treatment of cardiovascular disease. Cells are treated with a test agent and modulation of the agent is correlated to level of cardiovascular disease biomarker in cells. The patent also cites protein purification and isolation methods from cells or tissues. One medium of cells are those cultured in serum containing medium. The patent quotes "In one embodiment, the cells can be cultured in serum containing medium: The volume of the medium can be reduced using 3k MWCO Vivaspin columns (GE Healthcare Life Sciences), then can be reconstituted with 1×PBS (Invitrogen®). Serum albumin can be depleted from all samples using **AlbuVoid** column (Biotech Support Group, LLC) following the manufacturer's instructions with the modifications of buffer-exchange to optimize for condition medium application."

Patent Citations

Narain, Niven Rajin, and Paula Patricia Narain. COMPOSITIONS AND METHODS FOR DIAGNOSIS AND TREATMENT OF PERVASIVE DEVELOPMENTAL DISORDER United States Patent Application 20150023949, Pub. Date 01/22/2015.

> The patent provides sample preparation research application to track modulators of disease processes and experiment data on metabolomics, transcriptomics, single nucleotide polymorphisms using sample preparation and an artificial intelligence based informatics platform. Serum containing media are reduced using columns, reconstituted with buffers and serum albumin is depleted using Biotech Support Group's **AlbuVoid™**. Secretome sample preparation of cultures and conditioned media requires reduction using dithiothreitol (DTT), alkylation using iodoacetamide and desalting using acetone precipitation. Pooled aliquots of tryptic digests are labeled using iTRAQ, and analyzed using liquid chromatography mass spectrometry.

Narain, Niven Rajin, Rangaprasad Sarangarajan, and Vivek K. Vishnudas. "INTERROGATORY CELL-BASED ASSAYS AND USES THEREOF." U.S. Patent No. 20,120,258,874. 11 Oct. 2012.

> Inventors Narain et al describe the invention of a cellular modeling system which develops molecular signatures allowing scientists to gain insight into the mechanisms of disease by providing information on tissue microenvironment. The invention created "hubs" which are drug discovery candidates obtained from a combination of "network biology, genomic, proteomic, metabolomic, transcriptomic, and bioinformatics tools and methodologies". Thus

information on disease diagnosis or intervention and insight into mechanisms of drug toxicity is obtained. The patient states: "In one embodiment, the cells can be cultured in serum containing medium: The volume of the medium can be reduced using 3k MWCO Vivaspin columns (GE Healthcare Life Sciences), then can be reconstituted withlxPBS (Invitrogen). Serum albumin can be depleted from all samples using **AlbuVoid™** column (Biotech Support Group, LLC) following the manufacturer's instructions with the modifications of buffer-exchange to optimize for condition medium applications."

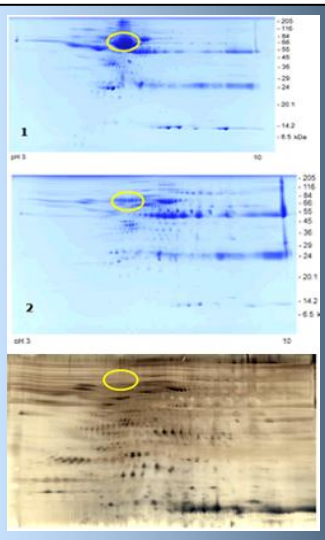
Albumin Removal Kits

• Unique surface chemistries, no antibodies • depletes Albumin 90-95%

AlbuVoid™

Selectively Voids Albumin, Binds Low Abundance Proteome

- Albumin voids in flow-through, >95%
- <30 minute protocol
- Low abundance enrichment equivalent or better than hexa-peptides or antibodies
- On-bead digestion protocols, efficient LC-MS workflows
- Disposable, cost-effective, no column regeneration or cross-contamination
- Mild elution maintains native structure with retained enzymatic, functional & bio-activities
- Species agnostic



2DE analysis of AlbuVoid™ treated sheep serum. Samples were reduced, alkylated and total protein normalized. The circled regions indicate the albumin zone. 1: Control. 2: AlbuVoid™ Eluate. Bottom: AlbuVoid™ Eluate silver stain.

AlbuSorb™

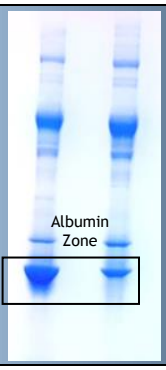
Selectively Binds Albumin

- Removes serum albumin >90%
- Economical small ligand surface architecture (not dye-based), bio-affinity performance
- Consumable, cost-effective, no column regeneration or cross-contamination
- Species agnostic
- Compatible with
  - LC-MS
  - Microarrays
  - Functional assays

Non-reduced SDS-PAGE profiles

Left lane: Normal human pooled serum control

Right lane: Flow-through from **AlbuSorb™**

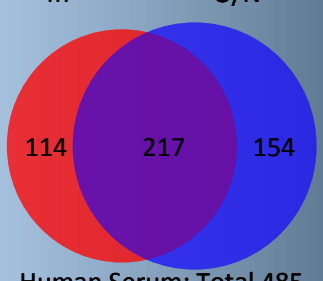


AlbuVoid™ LC-MS On-Bead

Selectively Voids Albumin, Binds Low Abundance Proteome before LC-MS use

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

Total LC-MS/MS Proteins identified at 2 digestion times: 4 hours (4h) & overnight (O/N), single 3 hour LC gradient.



AlbuSorb™ PLUS

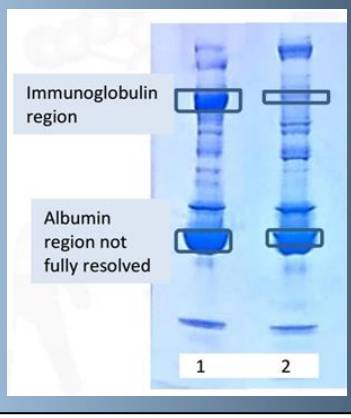
Selectively Binds Albumin & Immunoglobulin

- 400 µg total serum protein mass per prep
- > 85% Albumin, 85% IgG depleted from 25 µl serum

AlbuSorb™ combines with an optimized immobilized Protein A to create **AlbuSorb™ PLUS.** Unlike immuno-affinity, the surfaces utilized are disposable eliminating cycle to cycle variance and cross-contamination.

Lane 1: Human Serum Control

Lane 2: Serum after treatment with **AlbuSorb™ PLUS**



AlbuTrial™ Kit

Don't know which one to try? Try both. The AlbuTrial kit has 1 gram of **AlbuSorb™**, and 5 preps of **AlbuVoid™**

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