BIOTECH SUPPORT GROUP
Sample Prep that Matters

ALBUMIN REMOVAL REFERENCE APPLICATIONS

NOVEMBER 10, 2015

Introduction

The "omics" revolution demanded new and different sample prep separations that were not efficiently performed by conventional technologies. For years the protein separations toolkit was limited to liquid chromatography and gel electrophoresis. 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 most often denatured proteins which limited there use in applications which required the measurement of function, structure or bio-activity.

Two NuGel™ based 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

Albumin Removal Technology based on NuGel™ Silica Surface Chemistry

Through a proprietary polymer coating, 50 µm porous silica beads are crosslinked. This is the foundation of the NuGel™ surface chemistry. From this foundation, a library of bead architectures have been created. Each bead chemistry in the library presents a singular mixed-mode interaction; combining elements of ionic, aliphatic and aromatic hydrophobicity, and polymeric characteristics. One can think of these binding interactions in different terms; as general non-specific protein adsorbents, or as bead matrices with weak affinity or imperfect fit interactions. In this way, their binding behavior is very different from classical high affinity binding which demands near perfect fits. Finally, all derivative NuGel™ products were empirically characterized to meet the needs of the application; for example, AlbuVoid™ to selectively void (not bind) Albumin while capturing the majority of the remaining low abundance serum proteome on the bead.

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.

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 Biomarkers
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

Haiyan Zheng; Caifeng Zhao; Meiqian Qian; Swapan Roy; Absari Arpa; Matt Kuruc. Poster entitled "AlbuVoid™ Enrichment & On-Bead Digestion – Tackling The Challenges of Serum Proteomics". Poster at 63rd ASMS Conference on Mass Spectrometry, May 31- June 4, 2105.

Serum and plasma proteomics can be challenging for two reasons:

1) The presence of high abundance proteins; Albumin alone accounts for about 50% of the total protein mass, and

2) Serum and plasma as a whole presents a challenging proteolytic sample because of the large amount of glycoproteins; the carbohydrate modification blocking Trypsin access and efficiency.

We previously reported for AlbuVoid™, on-bead Trypsin digestion was equivalent or better than in-solution, with LC-MS/MS derived peptide and protein identifications comparing favorably with immuno-depletion. Such results were based on the optimal in-solution condition at pH 8. We herein report on a new product and method called **AlbuVoid™ On-bead LC-MS**, with optimized (patent pending) on-bead digestion conditions at pH 7.

Application Report – "AlbuVoid™ & On-Bead Digestion: Tackling the challenges of serum proteomics."

Biotech Support Group reports on a new application on **AlbuVoid™**, an albumin depletion reagent that selectively voids albumin and enriches the low abundance proteome. The application report obtains unique protein identification's from human and rat sera upon depleting albumin using **AlbuVoid™** in addition to providing a serum proteomic workflow. The **AlbuVoid™** proteomics workflow on albumin depletion has compatibility with quantitative label (i.e., isobaric tags for relative and absolute quantitation (iTRAQ)) and label-free LC-MS methods with minimal mis-cleavages. The application report provides data on immobilized ConA to enrich glycopeptides and enzymatic cleaving of glyco-bond producing glycoprotein fraction specific peptides. On bead digestions minimize proteolytic hydrolysis inconsistencies with seamless workflows; solution digests and C18 desalting are not required.
Download the application report from the Biotech Support Group website:
http://biotechsupportgroup.com/sites/default/files/AlbuVoid%20On-Bead%20Application%20Report.Rev4%20042015_0.pdf

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

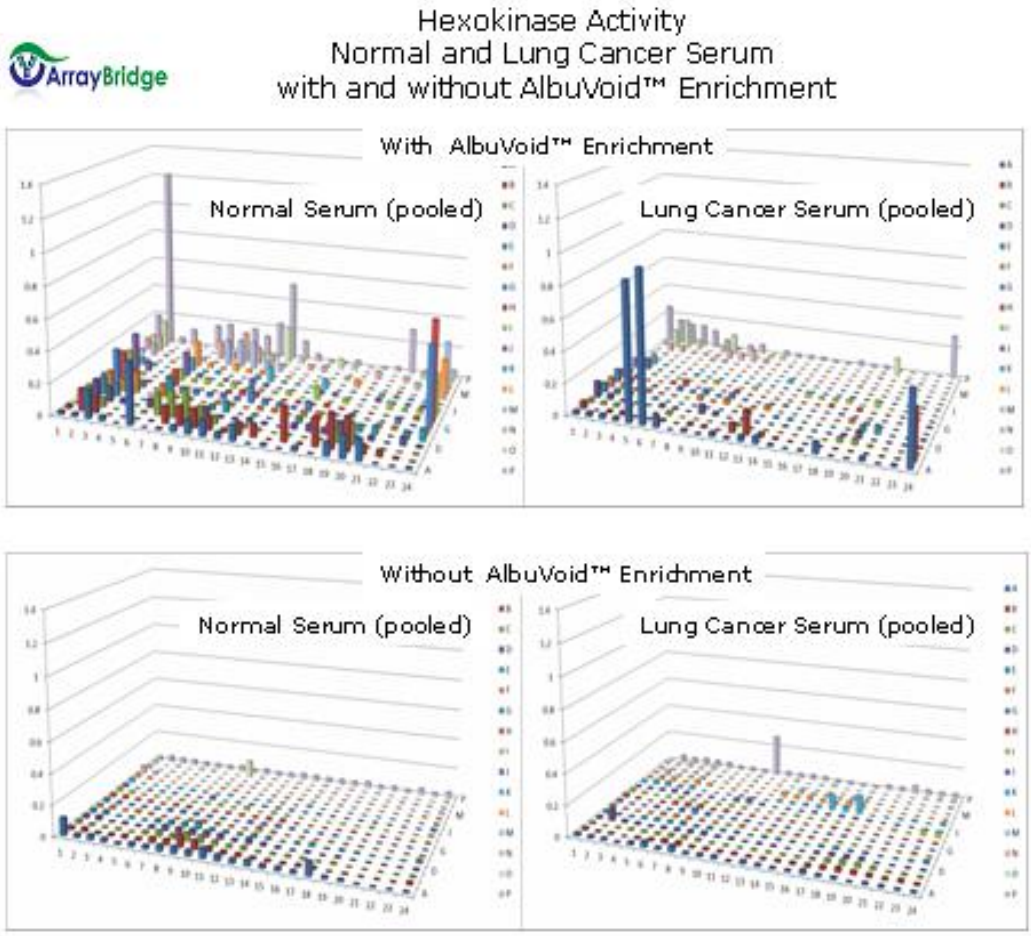
Using the new **AlbuVoid™ LC-MS On-Bead** product, we spectrally quantified over 200 total proteins, 21 of which were differentially observed as either over or under expressed in the cancer sera. These results support new efficiencies for serum proteomics. We solicit that such workflows will minimize 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

Discovery of Functional Serum Biomarkers Using **AlbuVoid™** Enrichment and the ArrayBridge PEP Profiling Platform. Personal communication, Xing Wang, Ph.D., ArrayBridge (St. Louis, MO), manuscripts in process.

Knowledge surrounding the functional annotation of the proteome is vitally important as the landscape of protein conformations is highly variable, each conformation may contribute to a unique functional activity. Sequence annotation alone cannot capture this vital information, so new strategies are necessary. Nevertheless, reconciling protein identifications to actual enzyme activities or functions has been subject to limitations in proteome separation and assay technologies. To overcome these inefficiencies in functional annotation, a top-down approach, starting with function, and ending with sequence and structural annotation is now available. The PEP technology, developed by ArrayBridge, uses a modified Two-dimensional Gel Electrophoresis to separate the proteome, without substantially compromising function. The isolated proteins are then electro-eluted from the PEP plate, and enzyme activities are measured systematically. This method thus provides a new functional dimension to explore the human serum proteome.

We report that by using **AlbuVoid™** as an upfront serum enrichment product, the features displayed from the PEP technology is substantially improved compared to the virtually featureless landscape observed without any such enrichment. As an example using Hexokinase profiling, the final result is that with **AlbuVoid™** enrichment, feature differences can be established between normal and cancer serum samples; such as would not be the case without **AlbuVoid™** enrichment.



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."

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.

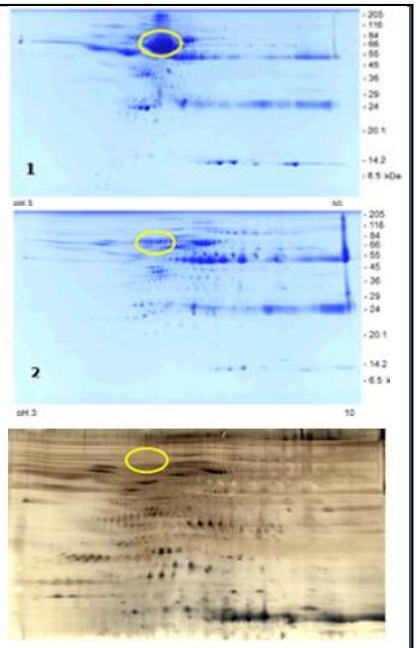
Narain, Niven Rajin, Rangaprasad Sarangarajan, and Vivek K. Vishnudas. "INTERROGATORY CELL-BASED ASSAYS AND USES THEREOF." U.S. Patent No. 20,120,258,874. 11 Oct. 2012.

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 application."

Albumin Removal Kits
Enrichment of Low Abundance Serum/Plasma Proteins

AlbuVoid™
Selectively Voids Albumin, Binds Low Abundance Proteome

- Albumin voids in flow- >95%
- Transferrin voids >99%
- <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. Gel 1: Sheep serum sample. Gel2: AlbuVoid™ Eluate. Gel 3: Same as 2 - AlbuVoid™ Eluate but restained with SilverQuest (Invitrogen) silver stain. The differences between the gels illustrate the efficiency of albumin removal, with no intrinsic pI or MW bias.

Product	Size	Item No.
AlbuVoid™	5 Preps	AVK-05
AlbuVoid™	10 Preps	AVK-10
AlbuVoid™	50 Preps	AVK-50

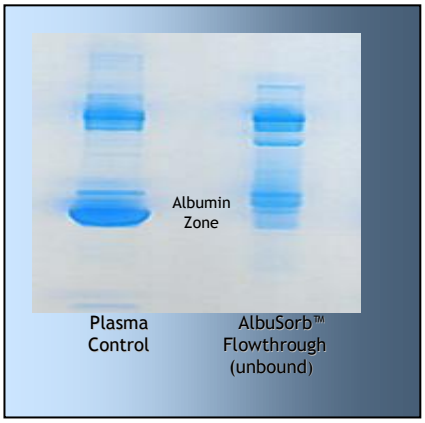
Based on 100-200 µl serum preps

Typical Performance	
Serum Sample Volume	100-200 µl
Albumin Removal	>95%*
LC-MS unique proteins	400-600
LC-MS unique peptides	3000-5000
Total Low Abundance Protein Recovery	>95%*

* Estimates based on SDS-PAGE visualization combined with Total Protein Assay.

AlbuSorb™
Selectively Binds Albumin

- Removes 30 mg albumin/ml, >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
  - Chemical
  - Functional proteomics



Product	Size	Item No.
AlbuSorb™	1 gm	A185-1
AlbuSorb™	6 gm	A185-6

1 gm processes 20 preps, 25 µl serum samples

AlbuVoid™ LC-MS On-Bead

Albumin depletion plus low abundance protein enrichment coupled with optimized on-bead digestion protocols for LC-MS serum and plasma proteomics

- Seamless workflows, unique proteolytic efficiencies
- Label, label free & glyco-compatible
- See page 5 of full catalog for more information and ordering

AlbuTrial™ Kit

Don't know which one to try? Try both. AlbuTrial™ kit is a combination of AlbuSorb™ and AlbuVoid™ with respective buffers.

Product	Item #
AlbuTrial™ Kit	AVS-05

The Kit includes:
1 Gram **AlbuSorb™** beads +
5 Preps **AlbuVoid™**