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AlbuVoid™

Albumin Depletion Plus Low Abundance Serum Protein Enrichment from Serum, Plasma, Tissues And Culture Media

- Albumin voids in flow-through >95%, with <30 minute bind/wash/elute protocol
- Low abundance enrichment equivalent or better than hexa-peptides or immuno-affinity
- Disposable, cost-effective, no column regeneration or cross-contamination
- Mild elution maintains tertiary structure and simple transfer to secondary analysis
- The eluted fractions retain their enzymatic and biological activity
- Works for all species tested including human, sheep, bovine, goat, rat, mouse, and calf.
- No molecular weight or pI bias
- For LC-MS, seamless On-bead protocols (BASP™) workflows and unique proteolytic efficiencies
    - No in-gel digests, no solution digests, no C18 desalting, more consistent, reproducible results
    - Compatibility with quantitative label (i.e., iTRAQ) and label-free LC-MS methods

AlbuVoid™ is an albumin depletion reagent kit, however the beads do not bind albumin It removes albumin from serum and plasma samples while concentrating low abundance proteins. The **AlbuVoid™** protocol uses mild buffers; the protocol conditions are very gentle so that native enzyme and functional activity is retained in elution fractions.

AlbuVoid™ derives from a silica-based library of individual imperfect fit polymeric ligands. The library was designed to facilitate weak binding of proteins, allowing for preferential displacement of the stronger bias binding proteins. Finally, **AlbuVoid™** method can deplete Albumin and enrich remaining serum sub-proteome without the use of antibodies.

The low abundance proteins which bind to **AlbuVoid™**, are eluted off without the albumin. Consequently, the low abundance serum proteins are enriched. It is ideal for applications involving discovery and targeted proteomics, enzyme assays, functional proteomics, protein profiling, protein arrays, 1D and 2D gel electrophoresis and LC-MS.

The **AlbuVoid™** beads have been adapted to a protocol specifically designed for LC-MS applications whereby the low abundance proteome adsorbed to the beads is proteolytically degraded to its peptide constituents. This is called Bead Assisted Sample Prep or BASP™; the protocol is included as an optional digest method.

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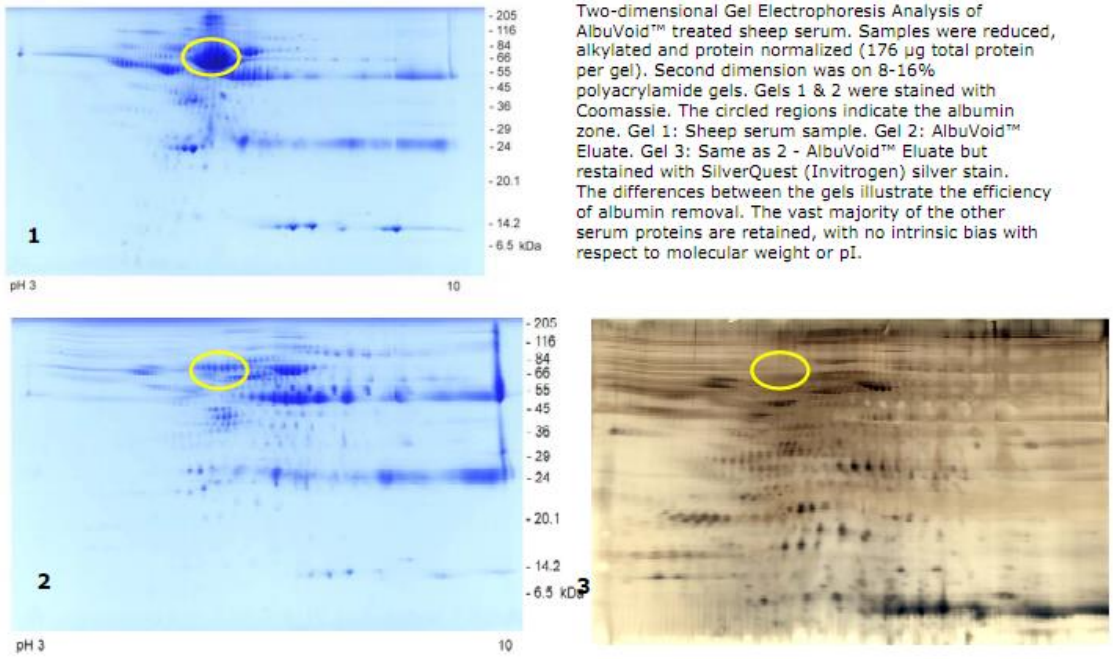

Two-dimensional Gel Electrophoresis Analysis of AlbuVoid™ treated sheep serum. Samples were reduced, alkylated and protein normalized (176 µg total protein per gel). Second dimension was on 8-16% polyacrylamide gels. Gels 1 & 2 were stained with Coomassie. The circled regions indicate the albumin zone. Gel 1: Sheep serum sample. Gel 2: 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. The vast majority of the other serum proteins are retained, with no intrinsic bias with respect to molecular weight or pI.

Product	Size	Total samples processed	Item No.
AlbuVoid™	5 Preps	5 x 200 µl samples	AVK-05
AlbuVoid™	10 Preps	10 x 200 µl samples	AVK-10
AlbuVoid™	50 Preps	50 x 200 µl samples	AVK-50
Note: Please contact sales@biotechsupportgroup.com for prices in bulk quantities.			

Albumin and IgG Removal Products
AlbuSorb™ PLUS and AlbuVoid™ PLUS are products that deplete both Albumin and IgG. For more information, go to:
https://www.biotechsupportgroup.com/AlbuVoid-PLUS-p/np-avk.htm
https://www.biotechsupportgroup.com/AlbuSorb-PLUS-p/apk285.htm

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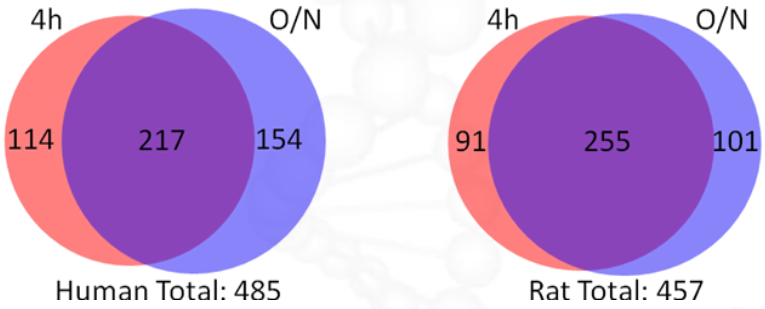
Comparison of 4 hour & Overnight Digestion Times

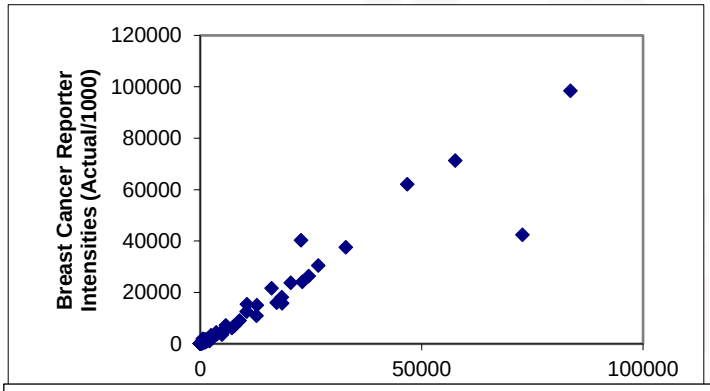
The total **AlbuVoid™** proteins were compared for human and rat sera at two different BASP™ digestion times, 4 hours and overnight (O/N). Note that many identified proteins overlap while certain populations of proteins were only observed in one or the other digest time (Zheng et al 2015).

Number of unique protein IDs after AlbuVoid™

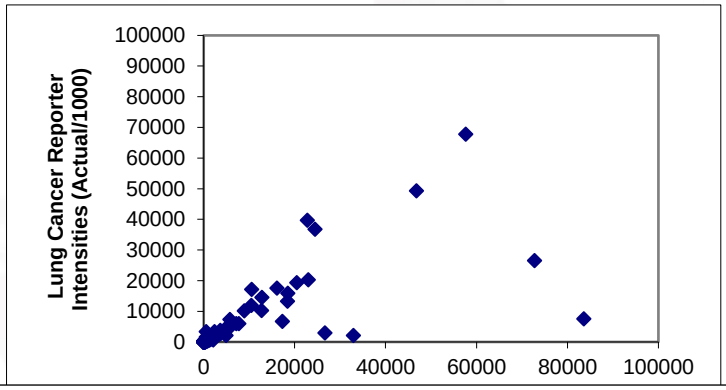


iTRAQ Labeled Peptides From Two Representative Disease Serum Samples

A. Breast Cancer Serum Proteins vs. Normal Serum Proteins.



B. Lung Cancer Serum Proteins vs. Normal Serum Proteins.



The reporter intensity signals were added together for each of the iTRAQ labeled peptides supporting the associated protein identification. Each of the additive peptide reporter intensities were then plotted for each protein comparing the following sample pairs: A. Breast Cancer Serum Proteins vs. Normal Serum Proteins. B. Lung Cancer Serum Proteins vs. Normal Serum Proteins. C. Breast Cancer Serum Proteins vs. Lung Cancer Serum Proteins. The linearity with only a few outliers demonstrates the reproducibility of the AlbuVoid™ with BASP™ digestion method for discovery applications.

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Items Required	5 Prep	10 Prep	50 Prep	Reagent
AlbuVoid™ Beads	0.25 gram	0.5 gram	2.5 grams	Supplied
Binding Buffer AVBB, pH 6.0	4 ml	7 ml	35 ml	Supplied
Wash Buffer AVWB, pH 7.0	5 ml	10 ml	50 ml	Supplied
Elution Buffer AVEB, pH 10	2 ml	4 ml	20 ml	Supplied
SpinX Centrifuge tube filters	5	10	50	Supplied

PROTOCOL – Based on processing 100-200 µl Serum

For best results – the lysate should be clear and free of colloidal material. We recommend first filtering through a 0.45 µm syringe-type filter before beginning the prep.

1. Weigh out 50 mg of **AlbuVoid™** beads in a spin-tube (0.45µ SpinX centrifuge tube filter from Corning).

2.Add 250 µl of **Binding Buffer AVBB .**Vortex for 5 minutes at room temperature followed by centrifugation at 3000 rpm. Discard the supernatant.

3.Repeat step-2

4.Condition by adding 200 µl of **AVBB** and 200 µl of the **Serum.** Vortex for 10 min and then centrifuge for 4 minutes at 10,000 rpm.

5. Remove the albumin enriched supernatant (Flow-Through) **FT**.

6. To the pellet add 500 µl of **Wash Buffer AVWB.** Vortex for 5 min and centrifuge for 4 minutes at 10,000 rpm. Discard the **Wash** filtrate.

7. Repeat Step-6. **The AlbuVoid™ bead is now enriched with albumin depleted low abundance proteins. As an option for LC-MS sample preparation, the bead assisted on-bead digestion protocol (BASP™) is provided, see box on next page. To elute the bound proteins, continue to step 8.**

8. To the pellet add 400 µl of **Elution Buffer AVEB.** Vortex for 10 min and centrifuge for 4 minutes at 10,000 rpm. Remove the filtrate as elution (albumin depleted proteins). The protein eluate is ready for further functional, proteomic or LC-MS analysis.

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Bead Assisted Sample Prep (BASP™), also known as on-bead digestion

8. Using **Wash Buffer AVWB**, prepare to 10mM of DTT concentration, and add 200 µl to the beads and vortex for 10 minutes and incubate for 30 minutes at 60C.

9. Cool the samples to RT, add suitable volume of Iodoacetamide to 20mM and incubate in the dark for 45 minutes

10.Centrifuge at 10,000 rpm (microfuge max setting) for 5 minutes, and discard supernatant. Rinse the bottoms of the spin-X tubes with 500 µl of 50% ACN, AVWB twice, to remove any traces of the filtrate.

11. Add 16 µg trypsin in 200 µl **Wash Buffer AVWB** to the beads and keep at 37°C for a minimum 4 hours to maximum overnight. Overnight is recommended to start with. In select targeted circumstances, 2 hours may be sufficient.

12. Centrifuge at 10,000 rpm (microfuge max setting) for 5 minutes, and retain peptide filtrate.

13.To further extract remaining peptides, add 300 µL 10% formic acid, vortex 10 min, centrifuge at 10,000 rpm (microfuge max setting) for 5 mins., and add this volume to the first volume.

14.Total is about 500µl. Prepare to desired final concentration. Store at -80 °C until LC-MS/MS.

References:

Serum

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

In this work, the investigators focused on depleting albumin from human serum samples using an albumin depletion and low abundance protein enrichment kit – AlbuVoid™, which enabled the detection of several low-abundance proteins. By employing an optimized protocol, enriched proteins known as biomarkers for various diseases were identified. 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.

Poillet-Perez, Laura, et al. "Autophagy maintains tumour growth through circulating arginine." *Nature* (2018): 1.

Autophagy is a cellular mechanism which captures intracellular components and delivers them to lysosomes, where they are degraded and recycled, helping cells survive during times of starvation. One *in vivo* model to study autophagy is whole-body deletion of the essential autophagy gene *Atg7* in adult mice which causes a systemic metabolic defect that manifests as starvation intolerance. In order to measure the systemic proteomic

response of such deletion in this study, AlbuVoid™ was chosen as one method to remove albumin and enrich the low abundance proteomes from serum.

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

Grubbs, J. K., et al. "Investigation of the efficacy of albumin removal procedures on porcine serum proteome profile1." (2015).

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 proteoforms, and how those can establish relationships to disease phenotypes, gene mutations, and dysregulated mechanisms.

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.

Cancer Proteomics Application Report
Poster Reprint First Presented At AACR Annual Meeting 2016 Conference, Held April 17-20, 2016 New Orleans, LA USA. The Commonality of the Cancer Serum Proteome Phenotype as analyzed by LC-MS/MS, and Its Application to Monitor Dysregulated Wellness Haiyan Zheng1; Caifeng Zhao1; Swapan Roy2; Devjit Roy MD; Amenah Soherwardy2; Ravish Amin2; Matthew Kuruc21Rutgers Center for Integrative Proteomics, Piscataway, NJ; 2Biotech Support Group LLC, Monmouth Junction, NJ
This investigation demonstrates 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. It suggests 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.

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.

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Cell Culture

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.

CONTACT US

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