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AlbuVoid™ PLUS

Albumin and IgG Depletion From Serum/Plasma for Proteomics

- IgG removal >90% (70-80% of total Immunoglobulins removed)
- Albumin removal >95%
- Seamless and simple < 1 hour protocol
- Low abundance enrichment equivalent to immuno-affinity
- Disposable, cost-effective, no column regeneration or cross-contamination
- Mild elution maintains tertiary structure and simple transfer to secondary analysis
- Low abundance protein and enzyme enrichment, suitable for all analytical platforms (i.e., LC-MS, Immunoassay)
- Species agnostic, validated on human, sheep, bovine, goat, fish, etc.
- No molecular weight or pI bias
- For LC-MS, optional 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

The classical plasma proteins generally fall into functional categories, forming the vast majority of the mid-to-high abundance proteome. In serum, these sub-proteomes by mass content are: Albumin 50-60%; Immunoglobulins 10-20%; Transport Proteins (Transferrin, Apo) 5-10%; Complement related Proteins 3-5%; Protease Inhibitors 2-5%; and all others 2-5%. While these sub-proteomes are required for normal body homeostasis, they nevertheless can become dysfunctional during acute-phase and chronic stimuli.

So, depending on the needs of the investigation, it can be valuable to consider that one or more of these categorical sub-proteomes is simply background noise whereby depletion is beneficial. While in other cases, these same categorical sub-proteomes might provide new data and information and consequently, should not be depleted. Different **AlbuVoid™, AlbuVoid™ PLUS and AlbuSorb™ PLUS** workflows support different proteomic biases as outlined in the following Table.

Products and digest conditions produce different sub-proteome windows of observation. Categorically the acute-phase sub-proteomes differentiated in disease may vary greatly from those associated with chronic sub-proteomes. So there is great benefit in having options to enrich or deplete one or more of these sub-proteomes. **AlbuVoid™ PLUS and AlbuSorb™ PLUS provide such options**

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LC-MS Spectral Data Analysis of Human Serum

	Approx. plasma conc.	AlbuSorb™ PLUS	AlbuVoid™ PLUS FASP	AlbuVoid™ PLUS BASP™ On-bead digest
Total Spectral Counts (SC)		**14456**	**23575**	**23389**
Total Protein ID (≥2 SC)		**224**	**467**	**350**
% SC Albumin	**50%**	**16%**	**5%**	**5%**
% SC Immuno-globulins	**20%**	**12%**	**8%**	**6%**
% SC Apolipoproteins	**4%**	**6%**	**5%**	**5%**
% SC Transport Proteins	**8%**	**22%**	**27%**	**23%**
% SC Protease Inhibitors	**6%**	**25%**	**9%**	**10%**
% SC Complement related	**5%**	**7%**	**26%**	**31%**
% SC Coagulation/ Fibrinolysis	**4%**	**2%**	**4%**	**5%**
% SC Other	**3%**	**10%**	**16%**	**15%**

BSG's Albumin and IgG Removal Kits offer many such options:

- The '**PLUS**' products substantially deplete Immunoglobulins through separations at the protein level.
- The variable regions of Immunoglobulins are extremely heterogeneous, generating a background noise across the full LC gradient. On-bead digestion (BASP™) with **AlbuVoid™** substantially reduces the influence of such Ig peptide features. So in addition to workflow simplicity, BASP™ can be advantageously utilized in targeted proteomic workflows whenever the target proteins do not require strong denaturing conditions.
- With the exception of Immunoglobulins whereby FASP generates many more spectral features, both strong denaturing conditions (FASP) and on-bead digest (BASP™) conditions produce similar protein profiles.
- Both Apolipoproteins and heavily glycosylated proteins (i.e., α_1-Acid Glycoprotein) bind poorly to **AlbuVoid™**. For quantitative studies within these classes of proteins, **AlbuSorb™ PLUS** is recommended.
- The Complement sub-proteome is especially enriched by **AlbuVoid™ PLUS**. The digest conditions may bias towards one or more functional sub-populations, likely due to conformational transitions and protein-protein interactions (i.e., Factor Bb, Properdin) that occur upon activation. This needs further investigation.
- The low abundance sub-proteome is enriched 5+ fold with **AlbuVoid™** and **AlbuSorb™ PLUS**.

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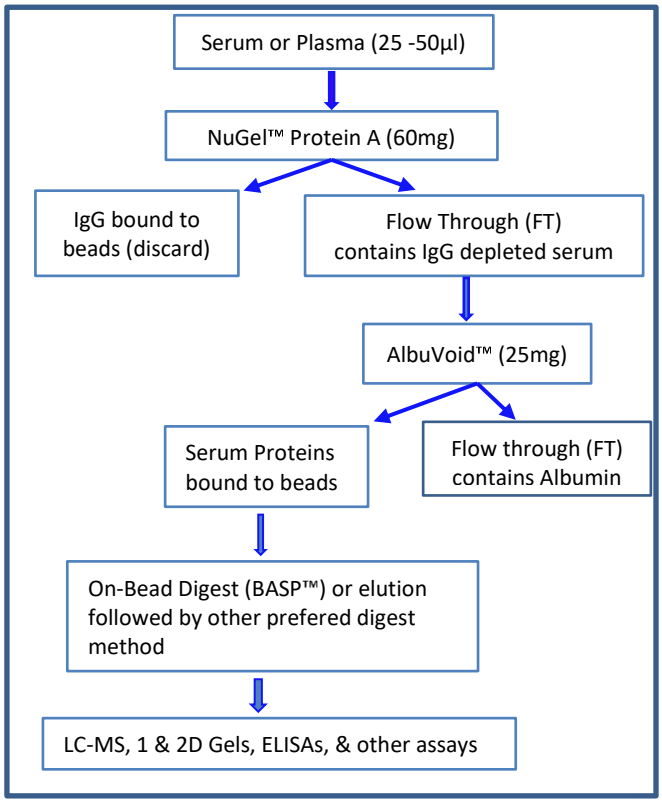
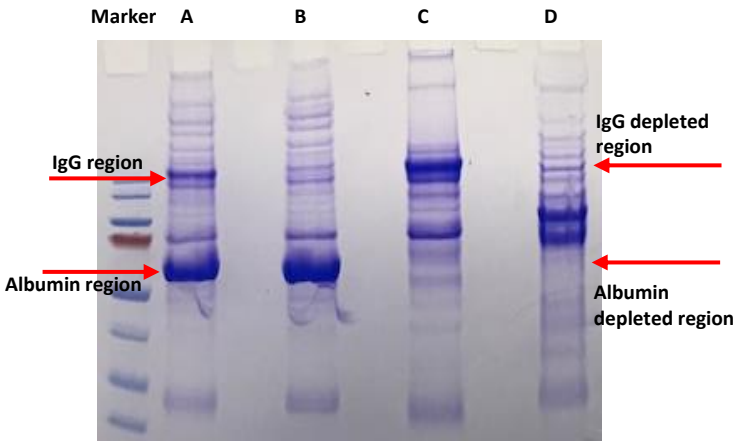
Typical Performance	AlbuVoid™ PLUS
Serum Sample Volume	25 – 50 µl
Albumin Removal	>95%
IgG Removal (most species)	>90%
Total Immunoglobulin Removal (most species)	70-80%
Recoverable Protein Mass	150 - 300 µg (Albumin + IgG depleted)
LC-MS/MS unique proteins (>2 Sp. Ct) (single 2 hr gradient)	300 - 500
LC-MS/MS unique peptide spectral counts (single 2 hr gradient)	20,000 – 30,000
For targeted proteomics, please contact technical services, as we have a knowledgebase of over 1000 serum proteins to help select the best method(s) for particular protein(s).	

Work Flow showing AlbuVoid™PLUS Protocol



SDS-PAGE: Comparison of three methods using Human Serum Sample



20µg of protein loaded on to 10% tris bis gel

A – Serum alone – shows IgG and Albumin regions
B - NuGel™ ProteinA treated serum – shows IgG depletion
C - AlbuVoid ™ treated serum – shows Albumin depletion
D – AlbuVoid™ PLUS treated serum – shows IgG and Albumin depletion

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Product	Size	# Serum Preps	Item No.
AlbuVoid™ PLUS Kit	5 preps	5, 25µl Serum samples	NP-AVK-05
AlbuVoid™ PLUS Kit	10 preps	10, 25µl Serum samples	NP-AVK-10

Items included in AlbuVoid™ PLUS kit

Items	Item No. NP-AVK-05	Item No. NP-AVK-10	Reagents
Kit#1 - IgG Depletion Kit			
NuGel™ Protein A Beads	300mg	600mg	Supplied
Buffer 1 (0.025M HEPES, pH 7.0)	5 ml	10 ml	Supplied
Spin-filter & tube assemblies	5	10	Supplied

Items	Item No. NP-AVK-05	Item No. NP-AVK-10	Reagents
Kit#2 - Albumin Depletion Kit			
AlbuVoid™ Beads	125mg	250mg	Supplied
Binding Buffer AVBB (0.05M HEPES, pH 6.0)	2 ml	4 ml	Supplied
Wash Buffer AVWB (0.05M HEPES, pH 7.0)	6 ml	12 ml	Supplied
Elution Buffer AVEB (0.25M Tris + 0.5M NaCl, pH 10.0-10.5)	2 ml	4 ml	Supplied
Spin-filter & tube assemblies	5	10	Supplied
DTT, Iodoacetamide, Trypsin and Formic Acid			Not Supplied

Additional Spin-Filters (low protein binding, 0.45 µm filter element) can be purchased separately, please inquire.

If there are any questions about compatibility or substitution with other buffers, please contact us.

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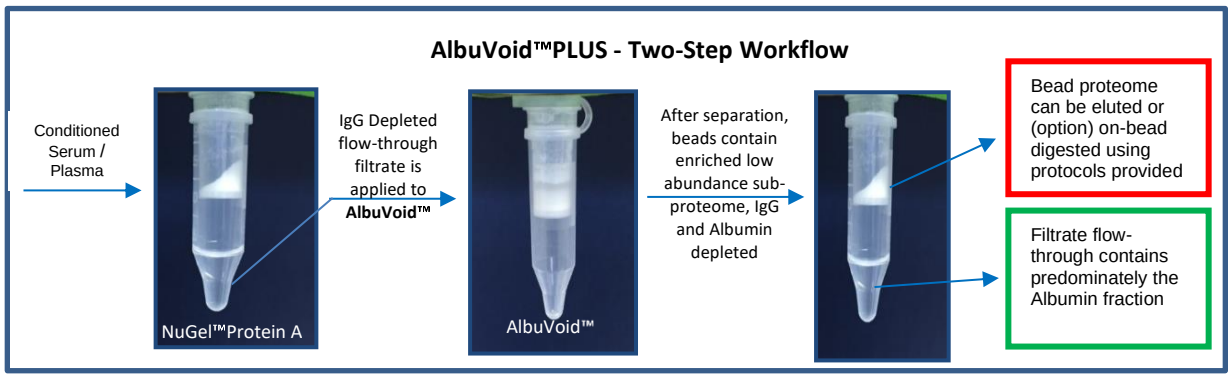

Protocol for IgG and Albumin depletion:

NuGel™ Protein A – IgG depletion Protocol (Kit# 1) – Based on processing 25µl Serum; maximum 50 µl can be used

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.

The centrifugation time may vary, adjust as necessary to get complete filtration through the beads.

The protocol can be scaled up or down proportionally to adjust for different volumes. The bead amount can be adjusted to accommodate more or less Albumin removal.

1. **Bead Conditioning.** Weigh out 60 mg of **NuGel™ Protein A** beads into the supplied microfuge spin-filters. Add 400 µl of **Buffer 1** to condition the **NuGel™ Protein A beads**. Vortex or mix thoroughly for 5 minutes at room temperature followed by centrifugation for 2 minutes at 5,000-10,000 rpm (2,000-8,000xg). Discard the filtrate.
2. **Sample Processing.** Add 250 µl of the **Buffer 1** to beads followed by 25-50 µl of serum to beads. Vortex or mix thoroughly for 10 min and then centrifuge for 4 minutes at 5,000-10,000 rpm (2,000-8,000xg). Keep the filtrate as it will be combined with the wash filtrate from step # 3.
3. For wash, add 200 µl of the **Buffer 1** to beads. Vortex or mix thoroughly for 10 min and then centrifuge for 4 minutes at 5,000-10,000 rpm (2,000-8,000xg). The 450 µl **filtrate contains serum proteins depleted of IgGs,** for application to **AlbuVoid™**.

AlbuVoid™ – Albumin Depletion Protocol (Kit# 2)

The IgG depleted filtrate from step 5, is prepared with **AlbuVoid™** to remove Albumin and enrich the remaining sub-proteome on the bead.

1. **Bead Conditioning.** Weigh out 25 mg of **AlbuVoid™** beads in a spin-filter. Add 150 µl of **Binding Buffer AVBB.** Vortex or mix thoroughly for 5 minutes at room temperature followed by centrifugation for 2 minutes at 5,000-10,000 rpm (2,000-8,000xg). Discard the filtrate. Repeat step-1.
2. Add 450 µl (IgG depleted filtrate) from **Nugel™ Protein A protocol.** Vortex or mix thoroughly for 10 min and then centrifuge for 4 minutes at 5,000-10,000 rpm (2,000-8,000xg). Discard the filtrate, containing predominantly Albumin.
3. To the beads, add 250µl of **Wash Buffer AVWB.** Vortex or mix thoroughly for 5 min and centrifuge for 4 minutes at 10,000 rpm. Discard the filtrate. Repeat Step (3). The **AlbuVoid™** beads contain the Albumin/IgG-depleted sub-proteome.

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4. **Bead Elution.** To the beads, add 200 µl of **Elution Buffer AVEB.** Vortex for 10 min and centrifuge for 4 minutes at 5,000-10,000 rpm (2,000-8,000xg). Recover the filtrate as elution (Albumin depleted proteins). The proteome eluate (0.25M Tris + 0.5M NaCl, pH 10.0-10.5) is ready for further functional, proteomic or LC-MS analysis.

As an option for LC-MS sample preparation, the bead assisted on-bead digestion protocol (BASP™) is provided below. The bead assisted on-bead digestion protocol (BASP™) is provided below. The digest buffer is Wash Buffer (0.05M HEPES, pH 7.0). Comparable buffers (0.02-0.5M, pH 6-7) can be used. Higher pH buffers are not recommended.

6. Using **Wash Buffer AVWB**, prepare to 10mM of DTT concentration, and add 100 µl to the beads and vortex for 10 minutes and incubate for 30 minutes at 60C.

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

8. Centrifuge at 5,000-10,000 rpm (2,000-8,000xg) for 5 minutes, and discard filtrate. Rinse the bottoms of the spin-filter tubes with 500 µl of 50% ACN, **Wash Buffer AVWB** twice, to remove any traces of the filtrate.

9. Add 8 µg trypsin in 100 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.

10. Centrifuge at 5,000-10,000 rpm (2,000-8,000xg) for 5 minutes, and retain digested peptides filtrate.

11.To further extract remaining peptides, add 150 µL 10% formic acid, vortex 10 min, centrifuge at 5,000-10,000 rpm (2,000-8,000xg) for 5 mins., and combine this volume with volume from step 10.

12.Total is about 250µl. Prepare to desired final concentration. Store at -80°C until LC-MS/MS.

Selection of AlbuVoid™ Reference Applications:

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.

Jing, Lun, et al. "PROTEOMIC ANALYSIS IDENTIFIED LBP AND CD14 AS KEY PROTEINS IN BLOOD/BIPHASIC CALCIUM PHOSPHATE MICROPARTICLE INTERACTIONS." *Acta Biomaterialia* (2021). Here, in a LC-MS/MS proteomic study, the article describes use of **HemoVoid™** and **AlbuVoid™** prior to LC-MS analysis, "…After **albumin depletion**, analysis of the significant deregulated proteins showed that **27 signaling pathways significantly changed in blood cells…**"

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

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 from mice and enrich the low abundance proteomes from serum.**

Zheng H, Zhao C, Qian M, Roy S, Arpa A, et al. (2015) AlbuVoid™ Coupled to On-Bead Digestion – Tackling the Challenges of Serum Proteomics. J Proteomics Bioinform 8: 225-230. The AlbuVoid™ bead enrichment is combined with a direct and seamless integration with Trypsin digestion, a method conventionally referred to as on-bead digestion. The digest time was evaluated as a parameter to identify whether different sub-populations of peptides and proteins can be observed by LC-MS

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analyses. Using 2 different 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 workflow efficiencies over other high abundance depletion and in-solution digestion workflows**.

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). 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.

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.
The article states "The **most dramatic difference for enzyme activity detection in using the AlbuVoid™ for serum protein enrichment was demonstrated** in the case of protease activity analysis**.** Compared with the direct serum proteinase measurement, both the levels and species of proteases were increased significantly in the enriched serum sample. …, **and it is necessary to use AlbuVoid™ to enrich these low level proteases to bring them to a high enough level to be detected**."

Cell Culture (BSA Removal)
Jenull, Sabrina, et al. "The histone chaperone HIR maintains chromatin states to control nitrogen assimilation and fungal virulence." *Cell Reports* 36.3 (2021): https://www.sciencedirect.com/science/article/pii/S2211124721008196#mmc1. The authors report a pivotal role for the HIR histone chaperone complex in modulating virulence of the human fungal pathogen *Candida albicans*. The article states for "Cell-free supernatants from 16 hours YNB-BSA (0.025% BSA) cultures grown at 30°C were used for Mass-Spec analysis. Collected supernatants were lyophilized and dissolved in 400 µl of water for **AlbuVoid™** treatment for albumin depletion…**Albumin-free enriched secretory proteome** was eluted from beads".

For a full list of Albumin Removal references, visit:
https://www.biotechsupportgroup.com/References-s/138.htm#albumin-depletion

CONTACT US

We welcome your questions and comments regarding our products.

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