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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
- Works for most species tested including human, sheep, rat, mouse, bovine
- On-bead protocols improve workflow and efficiency, especially suited to targeted proteomics
- Suitable for LC-MS, 1 and 2D Gels, ELISAs, Enzyme and other Functional Assays.

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. 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. 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.



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LC-MS Spectral Data Analysis of Human Serum

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 4+ fold with **AlbuSorb™ PLUS**.

	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 Immunoglobulins	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%

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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

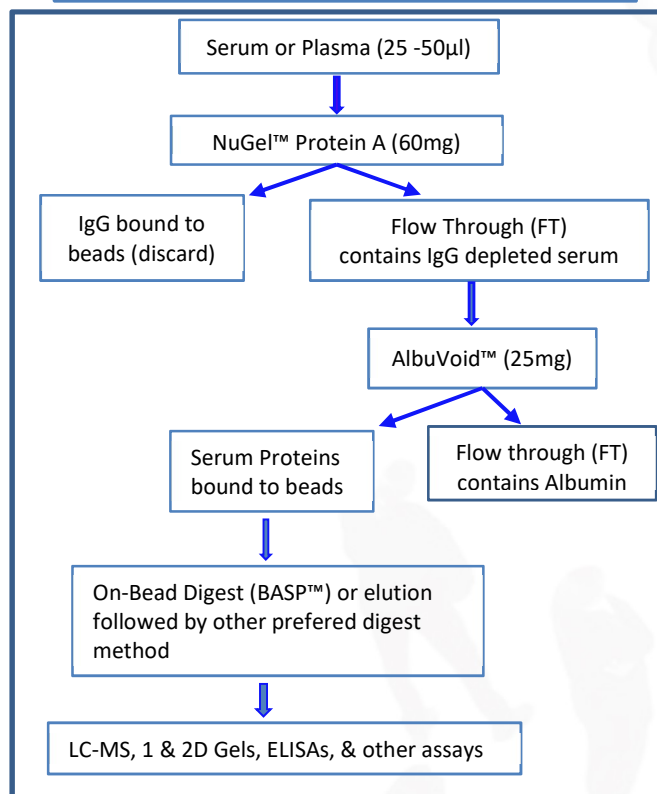
Items included in AlbuVoid™ PLUS kit

Items	Item No. NP-AVK-05	Item No. NP-AVK-10	Reagents
Kit#1 - IgG Depletion Kit			
NuGel™ Protein A Beads (NP)	300mg	600mg	Supplied
Buffer 1	5 ml	10 ml	Supplied
Spin-X Centrifuge tube Filters	5	10	Supplied

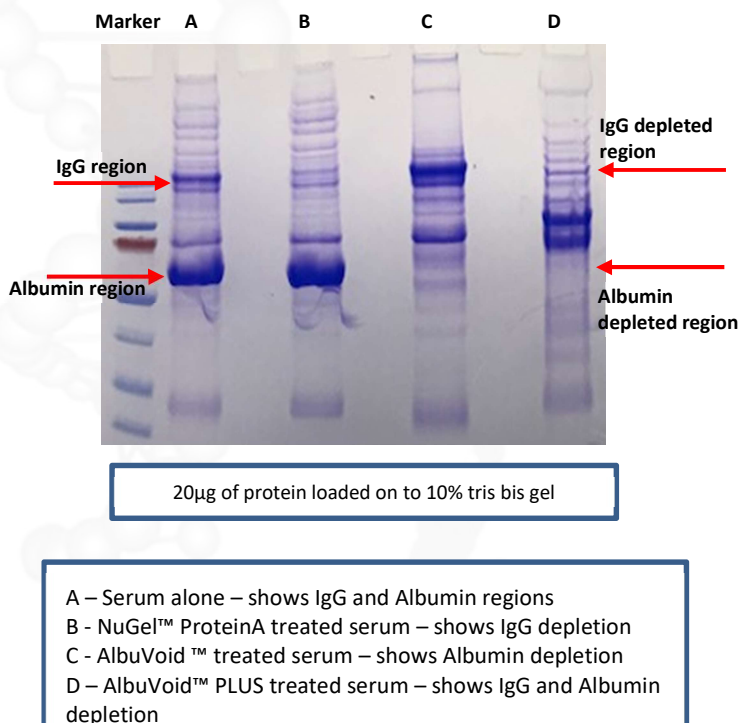
Items	Item No. NP-AVK-05	Item No. NP-AVK-10	Reagents
Kit#2 - Albumin Depletion Kit			
AlbuVoid™ Beads (AVK)	125mg	250mg	Supplied
Binding Buffer AVBB pH 6.0	7 ml	14 ml	Supplied
Wash Buffer AVWB pH 7.0	5 ml	10 ml	Supplied
Elution Buffer AVEB pH 10	2 ml	4 ml	Supplied
Spin-X Centrifuge tube Filters	5	10	Supplied
DTT, Iodoacetamide, Trypsin and Formic Acid			Not Supplied

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).	

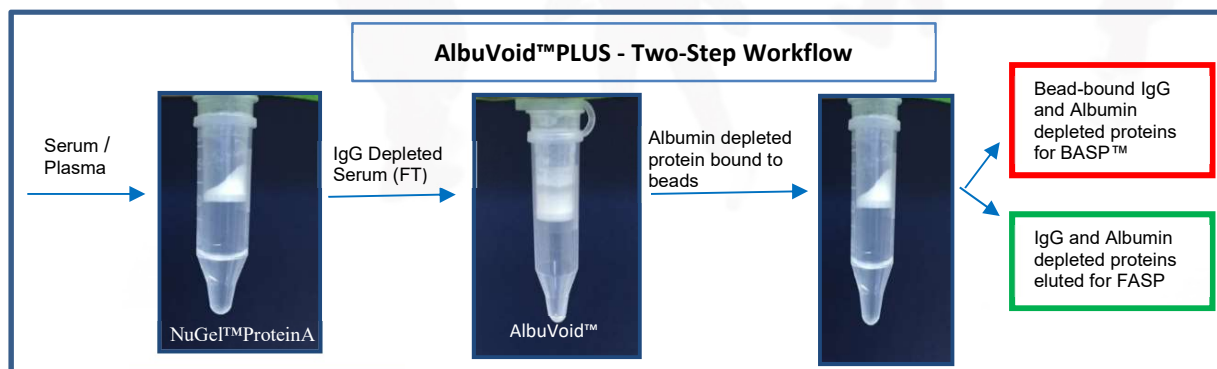
Work Flow showing AlbuVoid™PLUS Protocol



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



AlbuVoid™PLUS - Two-Step Workflow





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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 serum 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 60 mg of **NuGel™ Protein A** beads into the supplied microfuge spin-filters (0.45µ SpinX centrifuge tube filter).
2. Add 400 µl of **(Buffer 1)** to condition the **NuGel™ Protein A beads**. Vortex for 3 min. Centrifuge for 3 minutes at room temperature at 5000 rpm. Discard the filtrate.
3. Add 250 µl of the **(Buffer 1)** to 25-50 µl of serum to **Step 3**. Vortex for 10 minutes. Centrifuge for 4 minutes at 10,000 rpm.
4. For wash, add 100 µl of the **(Buffer 1)** to beads. Vortex for 10 minutes.
5. Centrifuge for 4 minutes at 10,000 rpm, 350 µl **filtrate contains serum proteins depleted of IgGs**.

The IgG depleted filtrate is now ready to for application to **AlbuVoid™** Step 3 below.

AlbuVoid™ – Albumin Depletion Protocol (Kit# 2)

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

1. Weigh out 25 mg of **AlbuVoid™** beads in supplied spin-filter (0.45µ SpinX centrifuge tube filter).
2. Add 250 µl of **Binding Buffer AVBB**. Vortex for 5 minutes at room temperature followed by centrifugation for 3 minutes at 5000 rpm. Discard the supernatant. Repeat this step again.
3. Add 175 µl **Binding Buffer AVBB** to the 350 µl from **NuGel™ Protein A protocol**, from Step 5 above. Vortex for 10 min and then centrifuge for 4 minutes at 10,000 rpm. Discard the Flow-Through fraction.
4. To the beads, add 250µl of **Binding Buffer AVBB**. Vortex for 5 min and centrifuge for 4 minutes at 10,000 rpm. Discard the filtrate.
5. Add 250µl of **Wash Buffer AVWB**. Vortex for 5 min and then centrifuge for 4 minutes at 10,000 rpm. Discard the wash filtrate.
6. The beads contain the Albumin/IgG-depleted sub-proteome. Options for digest include on-bead (BASP™); the protocol that follows, or other preferred digest conditions (i.e., FASP).

*For digest conditions or any other secondary treatment or analyses other than on-bead digestion, elute the bead-bound proteome by adding 200µl of **Elution Buffer AVEB**. Vortex for 10 min and centrifuge for 4 minutes at 10,000 rpm. The proteome eluate is ready for further functional or LC-MS analysis.*



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Sample Prep for Digestion [Options: **BASP™** or **FASP** described]

On-Bead Digest – **BASP™** (Bead-assisted Sample Prep)

1. Add 10mM of DTT in 100 μ l **Wash Buffer AVWB** to the beads and vortex for 10 minutes and incubate for 30 minutes at 60C.
2. Cool the samples to RT, add suitable volume of Iodoacetamide to 20mM and incubate in the dark for 45 minutes
3. Centrifuge at 10,000rpm for 4 minutes and discard the filtrate.
4. Rinse the bottoms of the spin-X tubes with 500 μ l of 50% ACN, **Wash Buffer AVWB**, to remove any traces of the filtrate.
5. Add 8 μ g trypsin in 200 μ l **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.
6. Centrifuge at 10,000rpm for 4 minutes and collect the filtrate.
7. Add 150 μ l of 10% formic acid to extract further peptides, vortex for 10 minutes and centrifuge at 10,000rpm for 4 minutes. Combine the filtrate (Total 350 μ l).
8. Dry the unused filtrate and store at -80°C. The sample is ready for LC-MS

Suggested **FASP** Method (Filter-Aided Sample Prep)

1. Use 35 μ l of elution and add 3.5 μ l 10X stock buffer (1M Tris pH 7.6, 1% Lauryl Dodecyl Sulphate) and 3.5 μ l from concentrated 1M DTT for final 100mM DTT reduction reaction.
 2. Heat the sample for 5 min at 60C, then cool to RT
 3. Samples were then diluted 1:2 with urea buffer (8M urea, 20mM methylamine hydrochloride, 100mM Tris-HCL pH 8.3) and centrifuged through Amicon Ultra 0.5ml centrifugal filters, regenerated cellulose, at 10,000 rpm for 15 minutes.
 4. Then rinse repeated with 200 μ l of urea buffer by spinning 10,000 rpm for 15 minutes.
 5. Add 100 μ l of 22.5mM iodoacetamide, in urea buffer, incubate at RT in dark for 40 minutes, spin at 10,000 rpm for 15 minutes.
 6. Add 200 μ l of urea buffer and spin at 10,000rpm for 15 minutes, repeat 3 x.
 7. Add 200 μ l of 50mM ammonium bicarbonate and spin at 10,000rpm for 15 minutes, repeat 3 x.
 8. Transfer filter to new collection tube.
 9. Proteins were digested with 2 μ g of trypsin in 75 μ l 50mM ammonium bicarbonate overnight in 37°C. Shake for 10 minutes and place samples in 37°C overnight.
 10. Spin at 10,000 rpm for 15 minutes.
 11. Add 200 μ l of 50mM ammonium bicarbonate, repeat spin at 10,000rpm for 15-20 minutes. (Total 475 μ l). Make sure to discard filtrate before it overflows and touches filter.
- The sample is ready for LC-MS.



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References

Zheng H, Soherwardy A, Roy S, Kuruc M, Avadhani S. AlbuVoid™ Enrichment & Antibody Depletion – Tackling the Challenges of Serum Proteomics Part II. Poster reprint first presented at 26th International Molecular Med TRI-CON, March 10-15, 2019, San Francisco, CA USA

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>

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

Roy, Swapan, and Matthew Kuruc. "Methods to Monitor the Functional Subproteomes of SERPIN Protease Inhibitors." *Functional Proteomics*. Humana Press, New York, NY, 2019. 41-54.

Mihara, Keisuke, et al. "Identification of Specific Protein Markers of Rheumatoid Arthritis in Synovial Fluid and Serum." *Journal of Hard Tissue Biology* 27.1 (2018): 55-58.

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. DOI 10.1186/s40364-017-0092-9

Zheng, H., et al. "AlbuVoid™ coupled to on-bead digestion-tackling the challenges of serum proteomics." *J Proteom Bioinformatics* 8.9 (2015): 225.

Zheng, H., et al. Poster reprint first presented at 14th Human Proteome Organization World Congress (HUPO 2015), held on September 27 – 30, 2015 entitled "New Proteomic Workflows Combine Albumin Depletion and On Bead Digestion, for Quantitative Cancer Serum".

CONTACT US

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