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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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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.

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%**

- 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**.

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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).	

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Work Flow showing AlbuVoid™PLUS Protocol

Serum or Plasma (25 -50µl)

↓

NuGel™ Protein A (60mg)

- IgG bound to beads (discard)
- Flow Through (FT) contains IgG depleted serum

↓

AlbuVoid™ (25mg)

- Serum Proteins bound to beads
- Flow through (FT) contains Albumin

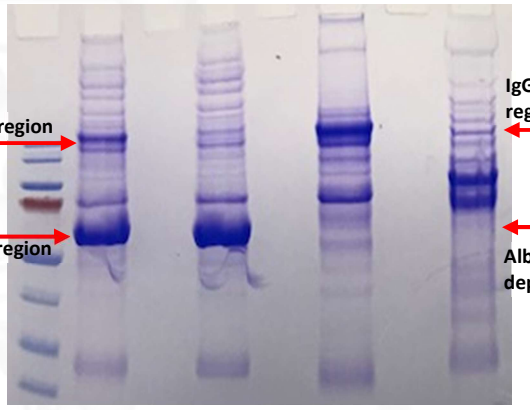
↓

On-Bead Digest (BASP™) or elution followed by other prefered digest method

↓

LC-MS, 1 & 2D Gels, ELISAs, & other assays

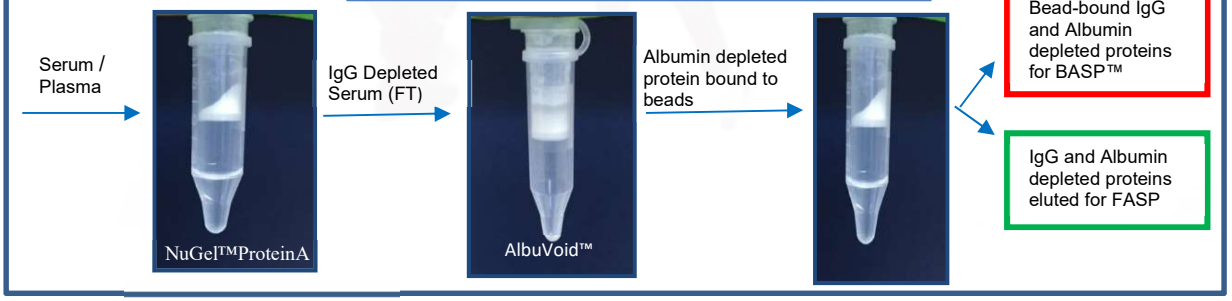
SDS-PAGE: Comparison of three methods using Human Serum Sample



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

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 µl **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 µl of 50% ACN, **Wash Buffer AVWB**, to remove any traces of the filtrate.
5. Add 8 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.
6. Centrifuge at 10,000rpm for 4 minutes and collect the filtrate.
7. Add 150 µl 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 µl).
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 35µl of elution and add 3.5µl 10X stock buffer (1M Tris pH 7.6, 1% Lauryl Dodecyl Sulphate) and 3.5µl 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 200µl of urea buffer by spinning 10,000 rpm for 15 minutes.
5. Add 100µl 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 200µl of urea buffer and spin at 10,000rpm for 15 minutes, repeat 3 x.
7. Add 200µl 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 2µg of trypsin in 75µl 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 200µl of 50mM ammonium bicarbonate, repeat spin at 10,000rpm for 15-20 minutes. (Total 475µl). Make sure to discard filtrate before it overflows and touches filter.

The sample is ready for LC-MS.

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CONTACT US

We welcome your questions and comments regarding our products.

Call 732-274-2866, 800-935-0628 (North America) Mon – Fri 9am-6pm EST.
Email sales@biotechsupportgroup.com
Mail 1 Deer Park Drive, Suite M, Monmouth JCT, NJ 08852, USA