AlbuSorb™ Product Extension combines Albumin and Immunoglobulin Depletion in a Consumable Format

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APPLICATION REPORT
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AlbuSorb™ Product Extension combines Albumin and Immunoglobulin Depletion in a Consumable Format

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Introduction and Objectives

From a foundational NuGel™ platform chemistry, a library of bead architectures has been created to support proteomic enrichment. These beads are general non-specific protein adsorbents, or stated another way - beads with weak affinity or imperfect fit interactions. Two of our 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. We now report on adding Immunoglobulin depletion as an extension to **AlbuSorb™**. A LC-MS/MS analysis on human serum revealed between 500-600 total proteins, many of which are qualitatively and quantitatively biased to sub-proteomes either depleted of Immunoglobulins, or not depleted of Immunoglobulins. **AlbuSorb™ PLUS** designates and distinguishes **AlbuSorb™** without immunoglobulin depletion, from **AlbuSorb™** with Immunoglobulin depletion. So **AlbuSorb™ PLUS** includes the collective advantages of all NuGel™ based products: *1) Consumable Use:* not derived from tissue sourced biologicals, no regeneration, cost-effective, no specialized instruments or HPLC, *2)Functional Integrity:* retains enzymatic and biological activity for functional and chemical proteomics, and 3) *Enrichment or Depletion:* strategies for both enrichment of low abundance proteomes, or depletion of high abundance proteins.

A significant challenge in blood based proteomics has been to overcome the analytical bias towards the most abundant proteins. Serum Albumin has the singular highest protein concentration in plasma and sera from all animals. To detect low abundance proteins, depletion of one or more high abundance proteins is common and desirable. To address this need, several commercial products incorporating either immobilized antibodies or combinatorial peptide libraries have been reported to either deplete high abundance proteins or enrich for low abundance proteins. We cite a report describing the differential effectiveness in MARS-14 spin cartridges in removal of 14 high abundance serum proteins, with the highest efficiency being for Albumin[1]. As albumin represents more than half of the protein mass in human serum, we have devised two strategies for albumin depletion**: AlbuSorb™** and **AlbuVoid™,** derived from the **NuGel™** silica-based platform. Each present a similar depletion efficiency but vary to some extent in their respective biases towards or against certain proteins,

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and sub-populations. Here we compare proteomic data derived from **AlbuSorb™**, **AlbuSorb™ PLUS** (which includes Immunoglobulin depletion), and **AlbuVoid™**.

NuGel™ Silica Surface Chemistry

Through a proprietary polymer coating, 50 µm porous silica beads are crosslinked and passivated. From this NuGel™ platform chemistry, a library of bead architectures has been created. Each bead chemistry in the library presents a 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. When conditions support protein binding saturation, progressive displacement allows the beads to bias for or against certain proteins. So in this manner, all derivative NuGel™ products were empirically characterized to meet the needs of the application. NuGel™ based kits include all necessary buffers to meet the application requirements.

- **AlbuSorb™** is used to selectively bind albumin from a wide range of mammalian sera.
- **AlbuSorb™ PLUS** combines albumin depletion with immunoglobulin depletion
- **AlbuVoid™** is used to deplete albumin by negative selection or voidance, and enrich for low abundance proteins on the bead.

Methods

The workflows follow standard protocols from each of the products: **AlbuSorb™**, **AlbuVoid™**, and **AlbuVoid™ LC-MS On-Bead**[2-4]. In brief, 25 µl (for **AlbuSorb™**), 50 µl (for **AlbuVoid™ LC-MS On-Bead**), and 200 µl (for **AlbuVoid™**) serum is prepared by adding a binding buffer, then applied to the beads, and washed. All steps are performed within a microfuge spin-filter format.

For **AlbuVoid™ LC-MS On-Bead,** after the final wash, reduction, alkylation and Trypsin digestion all take place on the bead. In the Table 1, we report the quantitative reproducibility for 3 parallel sample preps using the **AlbuVoid™ LC-MS On-Bead** method**.** For the rest of the data in this report, we considered only a 4 hour digest time to minimize proteolytic background as previously reported[5].

The LC-MS analysis was done at the Rutgers Center for Integrative Proteomics (Piscataway, NJ) with a single LC-MS/MS 3 hour gradient run. We used a nanoRSLC system interfaced with a Thermo Scientific™ Q Exactive™ HF (Thermo Scientific) instrument, using data-dependent acquisition with resolution of 60,000, followed by MSMS scans (HCD 30% of collision energy) of 20 most intense ions, with a repeat count of two and dynamic exclusion duration of 60 sec.

The LC-MS/MS spectral data was searched against the Human Ensembl databases using X!tandem (thegpm. org) with carbamidomethylation on cysteine as fixed modification and oxidation of methionine and deamidation on Asparagine as variable modifications using a 10 ppm precursor ion tolerance and a 20 ppm fragment ion tolerance. The searches were done using an in-House version of X! Tandem with protein filters set based on FPR supplied by the software: valid log(e) < -0.4, ρ =

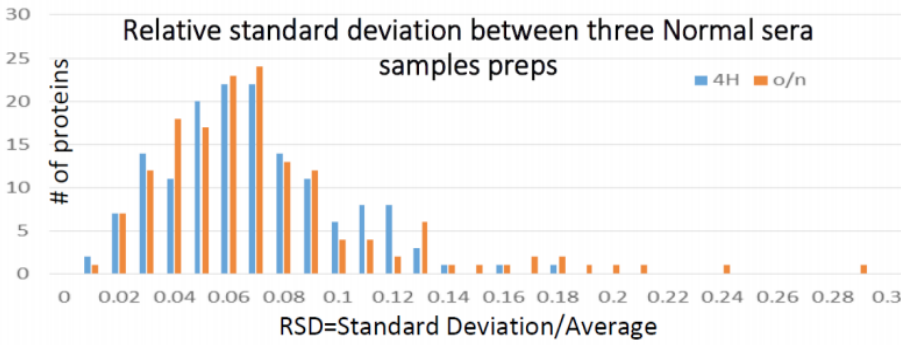
87, FPR = 0.72%. The peptides were filtered by loge<-2 and protein filtered by minimal number of peptide>2.

SDS-PAGE

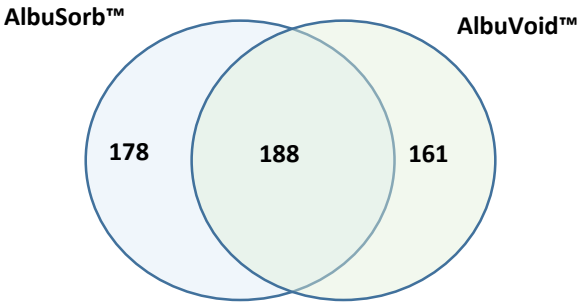
Criterion Precast Gels of 4-15% Tris-HCl (Bio-Rad) were used throughout. 20 µg of protein was loaded per lane, non-reduced conditions. Gel was placed in electro-apparatus with 1:10 ratio of 10x Tris/Glycine/SDS buffer, and separated at 150V for 80 minutes. Gel was stained with Coomassie.

Table 1. Reproducibility of triplicate AlbuVoid™ LC-MS On-Bead Preps

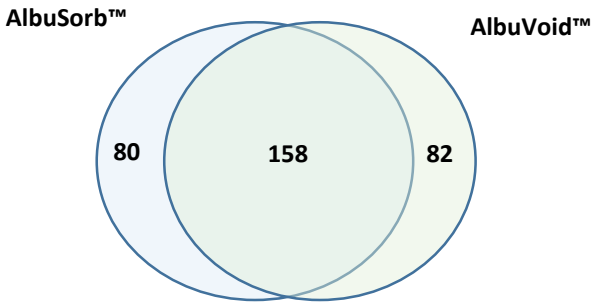
4 H: 4 hour digest
o/n: Overnight digest



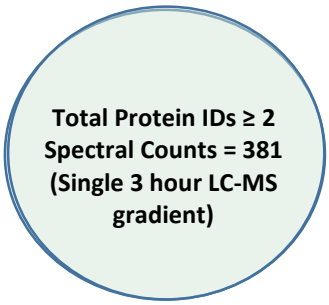
AlbuSorb™ vs. AlbuVoid™ Comparison



Total Protein IDs ≥ 1 Spectral Counts = 527 (Single 3 hour LC-MS gradient)



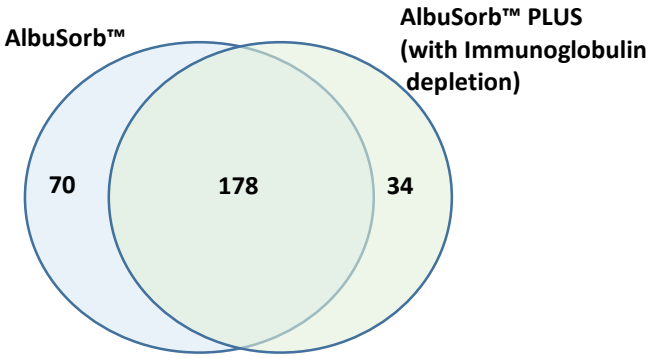
Total Protein IDs ≥ 2 Spectral Counts = 320 (Single 3 hour LC-MS gradient)



AlbuSorb™ + AlbuVoid™ split and combined into one pool

Quantitative Bias Comparison between AlbuSorb™, AlbuSorb™ PLUS and AlbuVoid™

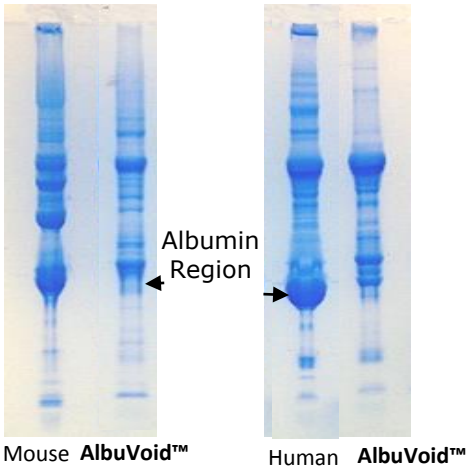
	AlbuSorb™	AlbuSorb™ PLUS
Total IG IDs 2+ Spectral Cts	80	58
Total IG % of Spectral Count	24%	9%
Total Albumin % of Spectral Count	12%	15%



Total Protein IDs ≥ 2 Spectral Counts = 282 (Single 3 hour LC-MS gradient)

NuGel™ based beads are Species Agnostic.

These non-reduced SDS-Page profiles show that albumin removal is specific, and suitable for both mouse plasma and human serum with equal efficiency.



Protein IDs with +++ Bias Towards one or the other prep	AlbuSorb™ Flowthrough (Unbound) Spectral Count	AlbuVoid™ Bead-Bound Spectral Count
A2M	1375	91
SERPINA1	519	59
ALB	1561	99
HP	315	28
IGHG2	308	27
PLG	7	77
C5	11	125
C6	1	69
HRG	4	65
CFI	2	48
IGHG4	140	19
IGFALS	0	46
SERPINF1	0	45
SERPINA4	0	45
AGT	62	4
F12	0	16
GC	58	0
CPB2	1	31
FBLN1	1	17
SERPINA10	0	23
CLEC3B	0	18
C1QB	0	26
C8G	0	17
HABP2	0	15
RBP4	32	0
CD5L	19	0
F5	2	50
C1QC	0	17
APOC1	0	27
AFM	35	0
IGFBP3	0	22
SERPINA6	27	0
LUM	24	0
AZGP1	22	0
ATRN	21	0
LRG1	19	0
SERPINA7	17	0
TOTAL	12203	8969

AlbuSorb™ vs. AlbuSorb™ PLUS Comparison

1: Human Serum Control (25 µl Serum + 250µl Buffer)

Immunoglobulin region

2: **AlbuSorb**™ Flow-Through

Analysis by gel estimation & LC-MS Spectral Counts

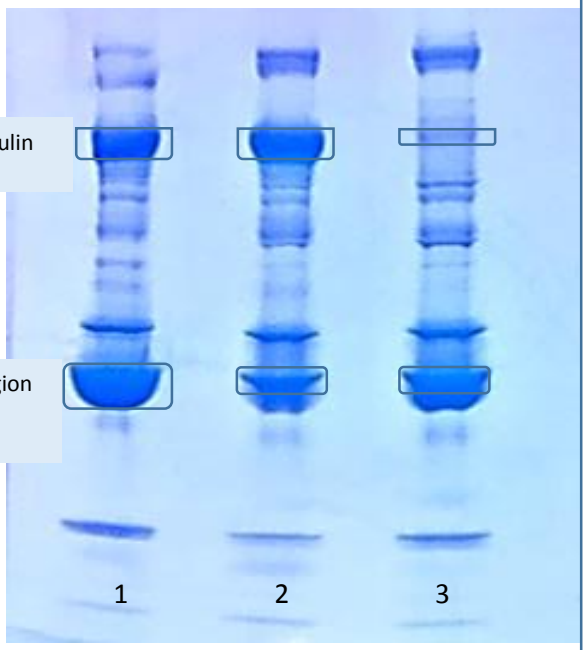
Albumin <10%, 90+%+ removal

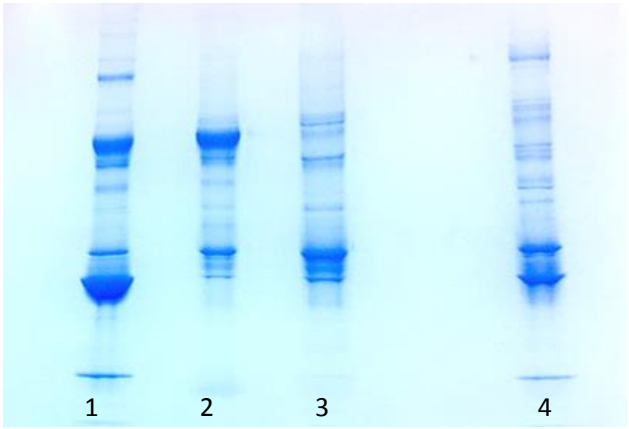
Albumin region not fully resolved

3: **AlbuSorb™ PLUS** Flow-Through

Analysis by gel estimation & LC-MS Spectral Counts

Albumin <15%, 80+%+ removal





Comparison of AlbuVoid™, AlbuVoid™ with Immunoglobulin depletion, and combined AlbuSorb™ and AlbuVoid™, both with Immunoglobulin depletion.

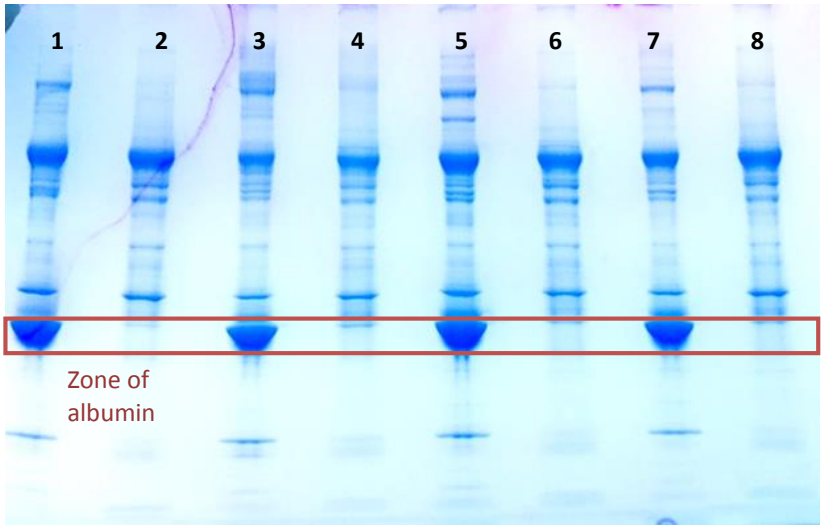
1: Human Serum Control (50 µl Serum + 150 µl Buffer)

2: **AlbuVoid**™ Eluent, standard protocol

3: **AlbuVoid**™ Eluent, with initial treatment of Protein A before AlbuVoid™

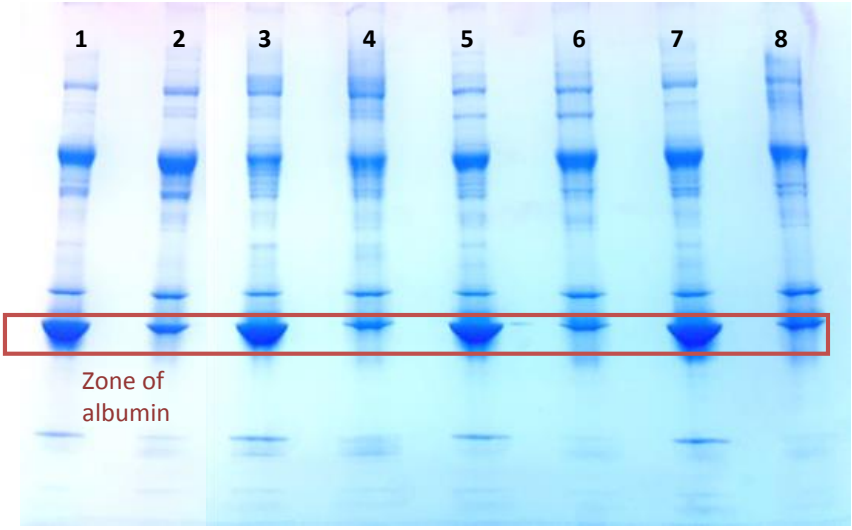
4. **AlbuVoid**™ Eluent, with initial treatment of Protein A before AlbuVoid™, combined 50:50 with **AlbuSorb™ PLUS** (AlbuSorb™ combination with Immunoglobulin depletion).

Cancer Sera Comparison of AlbuSorb™ and AlbuVoid™



Before and After AlbuVoid™

1: Normal pooled serum control
2: Elution from normal serum
3: Breast cancer pooled serum control
4: Elution from breast cancer serum
5: Lung cancer pooled serum control
6: Elution from lung cancer serum
7: Pancreatic cancer pooled serum control
8: Elution from pancreatic cancer serum
Note: All samples are from human female ages 40-60



Before and After AlbuSorb™

1: Normal pooled serum control
2: Flow-through from normal serum
3: Breast cancer pooled serum control
4: Flow-through from breast cancer serum
5: Lung cancer pooled serum control
6: Flow-through from lung cancer serum
7: Pancreatic cancer pooled serum control
8: Flow-through from pancreatic cancer serum
Note: All samples are from human female ages 40-60

Conclusions

> AlbuSorb™ combines with an optimized immobilized Protein A to create **AlbuSorb™ PLUS**. Unlike immuno-affinity, the surfaces utilized are disposable eliminating cycle to cycle variance and cross-contamination. Both **AlbuSorb™** and **AlbuSorb™ PLUS** are supplied as a powder, with all buffers necessary in kit formats. Simply weigh, centrifuge and/or filter, and recover the albumin depleted serum in the supernatant.

> An alternative depletion strategy uses **AlbuVoid™**. Because of its specialized voiding properties, **AlbuVoid™** negatively selects, or voids out high abundance proteins in serum – particularly albumin, while improving the resolution of less abundant serum proteins. Another novel feature of using **AlbuVoid™** is that on-bead digestion of the remaining serum proteome can produce unique proteolyic sub-populations with simpler workflows than using in-solution methods[5].

> For all of the albumin depletion methods, the protocols can be scaled up or down proportionally to adjust for different serum volumes. The bead amount can be adjusted to accommodate more or less albumin removal. Not beholden to biologically derived binding motifs, the chemically derived beads are species agnostic, and work with equal efficiencies towards human, mouse, rat, sheep, bovine and rabbit sera. With the versatility provided by the many separation strategies presented here, the user can optimize methods for discovery, or more targeted proteomic investigations.

> We maintain a database of serum proteins derived from all these methods, with quantitative characterizations using spectral counts and isobaric labels. Using **AlbuVoid™ LC-MS On-Bead**, we have begun an analysis of serum proteomes to characterize cancer sera phenotypes vs. normal/healthy ones. Based on early evidence, we believe it will be possible to apply phenotypic patterns to early detection, prognosis and treatment options, without regard to the primary tumor of origin, clinical stage, or tumor burden. In like manner, these methods can apply to any disease condition that might be monitored by serum profiling.

> Finally, all of the NuGel™ based bead chemistries maintain functional integrity of the enriched sub-proteomes suitable for functional proteomic analysis. As examples, we cite **AlbuVoid™** as an initial enrichment for the ArrayBridge PEP platform, and **NRicher™** series to observe and annotate soluble multiprotein complexes on a proteome scale[6,7]. So the products and methods just described can apply to workflows supporting LC-MS, ELISA, Antibody Arrays, 1 & 2DE, Western Blot, or functional analyses.

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