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AlbuSorb™ PLUS

Albumin + IgG Depletion From Serum or Plasma

- >400 µg total serum protein mass (> 85% Albumin, >85% IgG depleted) from 25 µl serum prep
- Affinity-type equivalence, virtually no cross-reactivity with other proteins
- Disposable, no column regeneration or cross-contamination
- Combines unique bead technology, not based on Blue-dye affinity, with optimized Protein A
- Mild conditions maintains structural integrity and simple transfer to secondary analysis
- Suitable for immunoassay, Western blot, 1 & 2D Electrophoresis, enzyme assay, LC-MS
- Tested species include human, sheep, bovine, rabbit, mouse, rat
- Validated in the automation compatible high-throughput DPX Technologies XTR tip format

Poly-electrolytes are polymers with repeating units of stationary charges. **AlbuSorb™** comes from a class of solid-phase, or surface-based, elastomeric poly-electrolytic surfaces that bind proteins through an empirically derived chemistry combining elements of polymer composition, cross-linking architecture and charge properties. 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. **AlbuSorb™ PLUS** is supplied as a powder. Simply weigh, centrifuge and/or filter, and recover the {albumin + Immunoglobulin} - depleted serum in the supernatant.

Gel Image: SDS-PAGE non-reduced, Criterion™ Tris.HCl (Bio-Rad) 4-15%

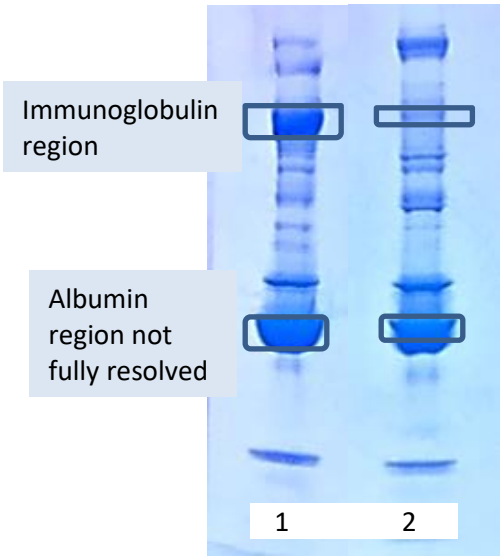
1: Human Serum Control (25 µl Serum + 250 µl Buffer)

2: **AlbuSorb™ PLUS** Flow-Through

Analysis by gel estimation & LC-MS Spectral Counts

Albumin <10%, 85+%+ removal

IG annotated <10%, 85+%+ removal



Product	Size	# Serum Preps	Item No.
AlbuSorb™ PLUS Kit	20 preps	20, 25 µl Serum Samples	APK285-20
AlbuSorb™ PLUS Kit	100 preps	100, 25 µl Serum Samples	APK285-100

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Typical Performance	AlbuSorb™	AlbuSorb™ PLUS
Serum Sample Volume	25 µl	25 µl
Albumin Removal	>90%	>85%
IgG Removal	-	>85%
Recovered Protein Mass	500-600 µg (Albumin depleted)	400-500 µg (Albumin + Ig depleted)
LC-MS/MS unique proteins (single 3 hr gradient)	350-400	350-400

LC-MS Proteomic Analysis of Serum (single 2 hr gradient)	Approx. plasma conc.	AlbuSorb™ PLUS
Total Spectral Counts (SC)		**14456**
Total Protein ID (≥2 SC)		**224**
% SC Albumin	**50%**	**16%**
% SC Immuno-globulins	**20%**	**12% (IgG mostly depleted)**
% SC Apolipoproteins	**4%**	**6%**
% SC Transport Proteins	**8%**	**22%**
% SC Protease Inhibitors	**6%**	**25%**
% SC Complement related	**5%**	**7%**
% SC Coagulation/ Fibrinolysis	**4%**	**2%**
% SC Other	**3%**	**10%**

SELECTIVE BINDING WORKFLOW

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Items	Included as part of Item No AVPS (AlbuTrial™ PLUS kit) 5 Preps	Item No APK285-20	Item No APK285-100	Reagent
AlbuSorb™ PLUS Beads	0.3 Gram	1.2 Gram	6.0 Gram	Supplied
Binding Buffer BB1 (0.05M K_2HPO_4 Dibasic, pH 7.5)	8 ml	30 ml	150 ml	Supplied
Microfuge Spin Filters	5	20	100	Supplied

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

PROTOCOL – Based on processing 25 µl Serum

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 **AlbuSorb™ PLUS** beads into the supplied microfuge spin-filters.

2. Add 400 µl of **Binding Buffer BB1** to condition the **AlbuSorb™ PLUS** beads. Shake it manually/ vortex for 3 min and then centrifuge for 2 minutes at 1,000 g's. Discard the filtrate.

3. Repeat step-2

4. As a requirement for albumin binding, add 250 µl of the **BB1 Buffer** and then add 25 µl of the serum to **Step 3**. Mix for 10 minutes on a rotating shaker.

5. Centrifuge for 4 minutes at 9,000 g's; **filtrate contains serum proteins depleted of albumin and Immunoglobulins.**

Note – when observing proteins on SDS-PAGE (4-15%), other high abundance proteins migrate to the same region as Albumin, and may not be fully resolved.

Scaleable and Versatile Protocol
The protocol 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.

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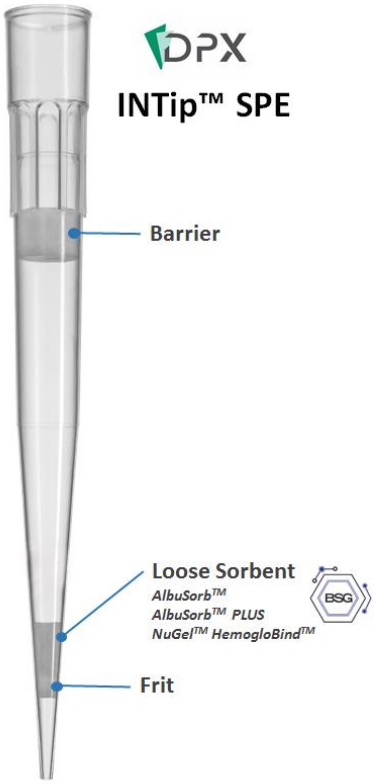
Validated in the high-throughput XTR tip format

The XTR tip format improves ease of use and scalability to process multiple samples in parallel, utilizing 96-well plates and automated liquid handlers. INTip™ SPE formats have been proven to be compatible with most automation platforms, i.e., Integra, Hamilton, etc. The **AlbuSorb™ PLUS** beads are loosely contained inside the XTR tips for a dispersive functionality that maximizes depletion efficacy.

A poster report is downloadable at:
https://www.biotechsupportgroup.com/v/vspfiles/templates/257/pdf/ASMSBSGDPXPoster.pdf

Please inquire for price and availability.



References

Poster Reports

"A Preliminary Investigation Using Targeted LC-MS Proteomic Methods Demonstrates Unique Serum Profiles of Hospitalized SARS-CoV-2 Patients"

https://www.biotechsupportgroup.com/v/vspfiles/templates/257/pdf/ASMSBSGLFPoster.pdf

For serum samples, targeted LC-MS is challenging, mainly due to the presence of highly abundant proteins. So it becomes critical to pair target peptides to sample depletion methods to best establish differentiated profiles between samples. In this preliminary investigation, Using AlbuSorb™ PLUS and other proprietary methods described, we characterize the functionality of the innate immune response in hospitalized Covid-19 patients, more precisely than current methods.

"AlbuVoid™ PLUS & AlbuSorb™ PLUS - Evaluating Different Windows of Observation Solves The Many Challenges of Serum Proteomics"

https://www.biotechsupportgroup.com/v/vspfiles/templates/257/pdf/PLUS%20Application%20Report%2007212019%20v1.pdf

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For serum, many proteomic enrichment strategies employ the use of immuno-affinity depletion to remove one or more high abundance proteins. Some common limitations of immuno-affinity however are high costs, and regeneration requirements which may result in a diminished and inconsistent performance. Because of these limitations, proteomic researchers need ways to enrich without immuno-affinity. This report considers the advantages of first reducing the influence of IgGs- a heterogeneous and proteolytically resistant class of proteins, along with Albumin depletion. Two products - AlbuVoid™ PLUS & AlbuSorb™ PLUS support depletion of both Albumin and IgG, through different strategies and workflows. Using LC-MS reporting metrics, the report highlights the serum sub-proteome bias characteristics of these products. Some examples of their selective utility for biomarker discovery in cancer are also presented.

Exosome

Chettimada, Sukrutha, et al. "Exosome markers associated with immune activation and oxidative stress in HIV patients on antiretroviral therapy." *Scientific Reports* 8.1 (2018): 7227.

Cerebrospinal Fluid

Gwenael Pottiez, Pawel Ciborowski. Proteomic Profiling of Cerebrospinal Fluid Expression Profiling In Neuroscience Neuromethods.2012;64:245-270

Synovial fluid

Happonen KE, Fürst CM, Saxne T et al. PRELP protein inhibits the formation of the complement membrane attack complex.Journal of Biological Chemistry.2012;287(11):8092-100

Serum

Valladolid-Acebes, Ismael, et al. "Lowering apolipoprotein CIII protects against high-fat diet–induced metabolic derangements." *Science Advances* 7.11 (2021): eabc2931.

Increased levels of apolipoprotein CIII (apoCIII), result in obesity-related metabolic derangements. Using mice, the researchers investigated mechanistically whether lowering or preventing high-fat diet (HFD)– induced increase in apoCIII, protects against the detrimental metabolic consequences. For Western blotting determination of circulating apoCIII, the article states, "plasma was albumin depleted using AlbuSorb according to the manufacturer's protocol (Biotech Support Group LLC) and resuspended in 0.1% (v/v) trifluoroacetic acid." The research concludes that lowering apoCIII protects against and reverses the HFD-induced metabolic phenotype by promoting physiological insulin sensitivity.

Nelson K, Wilkinson, S. et al., High resolution accurate mass spectrometry-based proteomics in ecotoxicology: SWATH-MS to detect differentially expressed plasma proteins in the amphibian toxicological model *Xenopus laevis*. Poster: Conference: PRIMO20, May 2019

Holmberg R, Refai E, Höög A.Lowering apolipoprotein CIII delays onset of type 1 diabetes. Proceedings of the National Academy of Sciences.2011;108(26):10685-9.

Tang MX, Ogawa K, Asamoto M. Effects of Nobiletin on PhIP-Induced Prostate and Colon Carcinogenesis in F344 Rats Nutrition and Cancer.2011;63(2):227-33

Holmberg, Rebecka Apolipoprotein CIII and Ljungan virus in diabetes 2010. Doctoral Thesis

Lu Q, Zheng X, McIntosh T Development of different analysis platforms with LC-MS for pharmacokinetic studies of protein drugs. Analytical Chemistry.2009;81(21):8715-23

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Cell/Tissue Culture Media
"AlbuSorb™ worked very well for us. We removed at least 90% of the albumin from our 10% FBS conditioned medium samples", states Joseph Sucic, University of Michigan.

Urine
Zubiri, Irene, et al. Diabetic nephropathy induces changes in the proteome of human urinary exosomes as revealed by label-free comparative analysis. Journal of Proteomics (2013).

Patent
Berggren, Per Olaf, Yang, Shao-Nian. 2012. Methods For Treating And/Or Limiting Development Of Diabetes.U.S. Patent 20120328630 Kind Code: A1, filed June 25, 2012, and issued December 27, 2012.

Albumin & IgG Removal products:
https://www.biotechsupportgroup.com/Albumin-Removal-s/307.htm

Lipid Removal Reagent and Clarification products:
https://www.biotechsupportgroup.com/Lipid-Removal-s/316.htm

Hemoglobin Removal products:
https://www.biotechsupportgroup.com/Hemoglobin-Removal-s/312.htm

CONTACT US

We welcome your questions and comments regarding our products.

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