User Guide

PureProteome™ Albumin Magnetic Beads

LSKMAGL10

FOR RESEARCH USE ONLY

Not for use in diagnostic procedures. Not for human or animal consumption.

Introduction

Human serum or plasma is a rich source of proteomic information, and is often interrogated for protein biomarkers of physiological and disease states. One of the overwhelming challenges in analyzing human serum (HS) is the wide concentration range of proteins present. Abundantly expressed proteins such as albumin make up approximately 50–70% of the total protein in serum/plasma, while protein biomarkers may be present at much lower concentrations (ng/mL to pg/mL). High abundant proteins are a challenge for analytical methods, such as two-dimensional gel electrophoresis and mass spectrometry, because they mask the lower abundant proteins of interest. It is critical for these applications that high abundant proteins are efficiently, reproducibly, and specifically removed from serum samples, enabling accurate analysis of the lower abundant proteins.

The PureProteome™ Albumin Magnetic Beads have been developed using an antibody ligand specific for human serum albumin. These magnetic beads provide a rapid, scalable, and reproducible means to deplete > 98% of albumin from serum and plasma samples, facilitating the detection and analysis of proteins of interest. PureProteome™ Magnetic Beads in combination with the PureProteome™ Magnetic Stand readily facilitate the depletion of multiple samples in parallel.

Materials Required

- For optimal performance, the PureProteome™ Magnetic Stand is recommended for use with PureProteome™ Magnetic Beads
- 2 mL microcentrifuge tubes
- Phosphate Buffered Saline (PBS)

Application Guidelines

Please read the User Guide completely before beginning the protocol.

Albumin Depletion from Serum Samples

This protocol is optimized for 25 µL of serum. It may be scaled up or down as required by available sample volumes.

1. Mix the bead suspension so that all of the beads are uniformly resuspended. To ensure consistent bead volume, continue to mix while pipetting.
2. Pipette 750 µL of the resuspended beads into a 2 mL microcentrifuge tube. Place the tube into the PureProteome™ Magnetic Stand and allow the beads to migrate to the magnet. Remove the storage buffer with a pipette and discard.
3. Wash the beads twice, using 500 µL of PBS for each wash. Disengage the magnet from the stand and vortex vigorously for 10 seconds. Re-engage the magnet and allow the beads to migrate to the magnet. Remove the buffer with a pipette and discard.
4. Dilute 25 µL of serum to a final volume of 100 µL with PBS.
5. Add the diluted serum sample to the beads. Incubate for 60 minutes at room temperature with continuous mixing or end-over-end rotation.
6. Place each tube back into the magnetic stand. Allow the beads to migrate to the magnet. Remove the depleted serum with a pipette. Transfer to a fresh tube and save.

   Note: This represents the albumin-depleted serum sample. The majority of the unbound proteins will be in the depleted serum/plasma sample.
7. To maximize recovery of the depleted serum sample, wash the beads 3 times, using 500 µL of PBS for each wash. After each wash, disengage the magnet and vortex vigorously for 10 seconds. Re-engage the magnet and allow the beads to migrate to the magnet. Remove the wash fraction with a pipette and **combine with the saved depleted serum.**
8. Store the depleted fraction at or below −20 °C for long term storage.

   Note: The sample may be concentrated and/or desalted prior to storage.

Elution of Proteins Bound to PureProteome™ Albumin Magnetic Beads (Optional)

The bound fraction of proteins may be analyzed by SDS-PAGE to ensure complete recovery of target protein(s) in the unbound depleted sample.

To elute the bound proteins, resuspend the beads 3 times using a minimum of 100 µL of 200 mM Glycine-HCl, pH 2.0 for each elution. After adding the Glycine-HCl, disengage the magnet and vortex vigorously for 10 seconds. Re-engage the magnet and allow the beads to migrate to the magnet. Remove the eluted fraction with a pipette and save.

Alternatively, the bound proteins may be eluted in a 1X SDS-PAGE Reducing Sample Buffer (RSB). Resuspend the beads 1 to 3 times using 100 µL of 1X RSB for each elution. After adding the RSB, disengage the magnet and vortex vigorously for 10 seconds. Remove tube and incubate at 70 °C for 10 minutes. Quickly place the tube back in the magnetic stand and allow the beads to migrate to the magnet. Immediately remove the eluted fraction with a pipette and save.

Note: The 12 kDa antibody ligand may be observed on the gel if the magnetic beads have been incubated in 1X RSB at 70 °C for 10 minutes.

Using Centrifugation for Concentration or Buffer Exchange (Optional)

Amicon® Ultra-4 3K centrifugal filter devices (not included; purchase separately) can be used for rapid concentration and buffer exchange/desalting of the sample. Typical processing time is 20–30 minutes to reduce the volume of depleted sample to 50–150 µL. A physical deadstop in the filter device prevents spinning to dryness and avoids potential sample loss. The concentrate is collected from the filter device sample reservoir using a pipettor. Concentration and buffer exchange/desalting of the depleted serum sample can be performed in the same device.

Alternatively, concentration and buffer exchange may be performed using a different method, such as protein precipitation.

Disposal

Used material may be discharged into sewer or industrial waste water systems if allowed by local regulations. Otherwise, collect and dispose according to federal, state, and local regulations.

Safety Data Sheet

Safety Data Sheets (SDS) are available on our web site. Go to SigmaAldrich.com and enter your catalogue number in the search box.

Specifications

Matrix	Mixture of polymer-coated inorganic beads covalently coupled to an anti-albumin ligand
Particle form	Spherical
Bead diameter	10 µm (nominal)
Storage	2–8 °C, do not freeze
% Depletion	> 98% Albumin; typical values are ~99%

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Performance

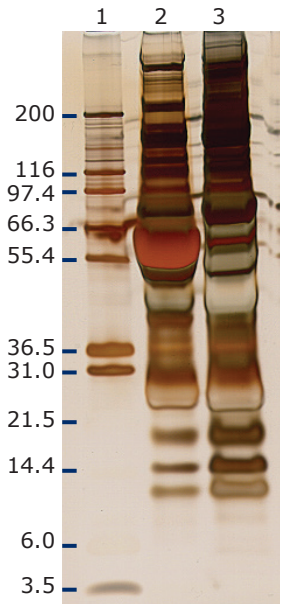
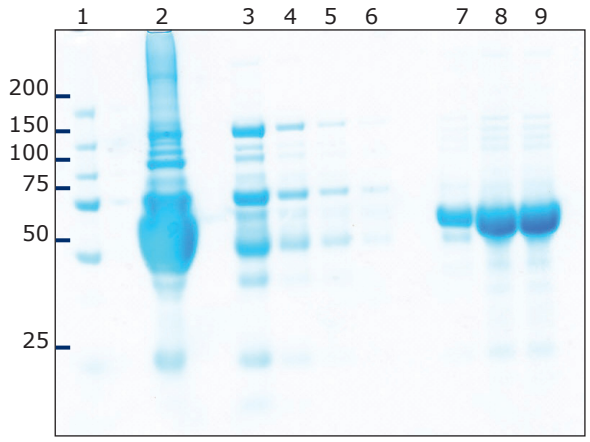


Figure 1: Removal of Human Serum Albumin (HSA) from Serum.

Proteins were resolved on 4–12% SDS-PAGE gel and silver stained. Human serum (25 µL) was depleted of albumin following the protocol described for the PureProteome™ Albumin Magnetic Beads.
Lane 1, molecular weight markers; lane 2, human serum (30 µg total protein); lane 3, human serum after depletion (30 µg total protein).



Lane	1	2	3	4	5	6	7	8	9
Sample	Molecular Weight Markers	HS	Unbound (depleted fraction)	Wash 1	Wash 2	Wash 3	Elution 1	Elution 2	Elution 3
Sample Volume (µL)	3	0.75	3	3	3	3	3	3	3

Figure 2: Human Serum (HS) Protein Analysis Pre- and Post-depletion.

Proteins were resolved on 4–12% SDS-PAGE gel and stained with Coomassie blue. HS (25 µL) was depleted of albumin following the protocol described for the PureProteome™ Albumin Magnetic Beads. The bound fraction was eluted from the magnetic beads using 3 × 100 µL additions of 200 mM Glycine-HCl, pH 2.0.

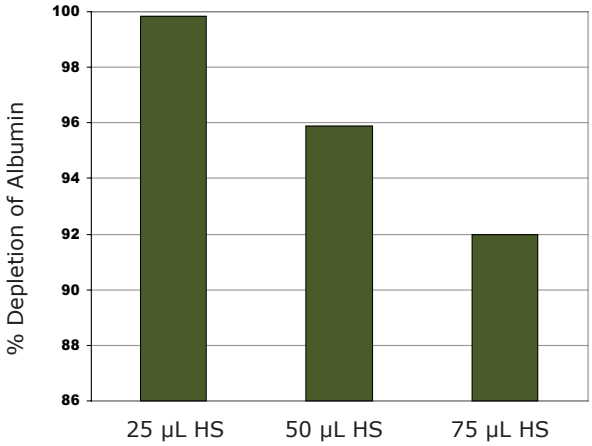


Figure 3: Depletion Efficiency of Human Serum Albumin (HSA) from Various Amounts of Human Serum.

Increasing amounts of human serum (25 µL, 50 µL, and 75 µL) were mixed with a fixed amount of PureProteome™ Albumin Magnetic Beads (750 µL of slurry or 150 µL of settled beads) and depleted as outlined in the protocol. The pre- and post-depleted HS samples were assayed by ELISA to calculate the percent depletion of HSA.

Product Ordering

Description	Qty/Pk	Catalogue No.
PureProteome™ Albumin/IgG Depletion Kit Contains magnetic beads, buffer concentrate, and Amicon® Ultra-4 devices	1	LSKMAGD12
PureProteome™ Albumin Magnetic Beads	10 mL	LSKMAGL10
PureProteome™ Magnetic Stand, 8-well	1	LSKMAGS08
PureProteome™ Magnetic Stand, 15 mL	1	LSKMAGS15
Amicon® Ultra-4 3K Centrifugal Device	8 24 96	UFC800308 UFC800324 UFC800396

Additional Products for Downstream Analysis

Description	Qty/Pk	Catalogue No.
Amicon® Ultra-0.5 3K Centrifugal Device	8 24 96	UFC500308 UFC500324 UFC500396
ZipTip® SCX Pipette Tip, 0.6 µL strong cation resin	8 96	ZTSCXS008 ZTSCXS096
ZipTip® C18 Pipette Tip 0.6 µL C18 resin	8 96 960	ZTC18S008 ZTC18S096 ZTC18S960
ZipTip® µC18 Pipette Tip 0.2 µL C18 resin	8 96 960	ZTC18M008 ZTC18M096 ZTC18M960
ZipTip® C4 Pipette Tip 0.6 µL C4 resin	8 96 960	ZTC04S008 ZTC04S096 ZTC04S960

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