Quick HSA/IgG Depletion Kit

Introduction

Serum and plasma are frequently used in clinical and pharmaceutical proteomics to discover early diagnostic biomarkers for therapeutic targets. Although these bodily fluid samples are non-invasive and simple to obtain, they contain some abundant proteins that disguise other protein components present in low amounts. The problem in discovering serum biomarkers is eliminating the abundant proteins while simultaneously uncovering and enriching the low-abundance ones. Proteins that are abundantly expressed, such as albumin and IgG, account for 70 percent of total protein in serum/plasma. In contrast, protein biomarkers may present considerably lower amounts (pg/ml). High abundance proteins pose problems for analytical procedures like one-dimensional and two-dimensional electrophoresis, high-performance liquid chromatography, and mass spectroscopy because they hide the less abundant proteins of interest. High abundant proteins must be effectively, reproducibly, and extracted explicitly from blood samples to correctly detect the lower abundant proteins. The selective removal of albumin from human samples (e.g., plasma, serum, urine, or cerebrospinal fluid) is necessary before performing proteome analysis, particularly when using mass spectrometry.

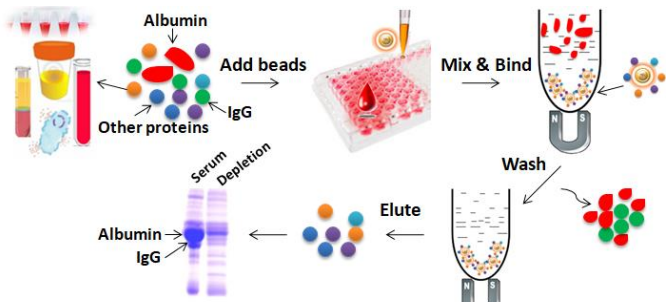
Although Cibacron Blue F3GA, an affinity dye ligand, was covalently bonded onto commercially available supports for HSA adsorption from both aqueous solutions and human plasma, it lacks selectivity. In immunoaffinity systems, anti-albumin antibodies are also employed. Protein A is often used to remove IgG. These techniques have reasonable specificity in general, but they are costly. Furthermore, because many of these systems are based on agarose resin or its derived column, their procedure for the enrichment of the less abundant proteins is time-consuming, unable to handle large samples volume in a short time and challenging to adapt to the automation system. At the same time, they are frequently performed by increasing the sample volume, requiring an additional protein concentration step. New albumin and IG removal methods with improved efficiency, homogeneity, and throughput are required to address the above issues.

BcMag™ Quick HSA/IgG Depletion Kit is based on our unique magnetic beads modified with our proprietary chemistry for quick and easy removal of both albumin and IgG from serum and plasma samples. The beads bind to all other proteins in the sample (except albumin and IgG) in particular buffer conditions, allowing the binding and release of all less abundant proteins but albumin and IgG. The enriched and eluted proteins can be used for downstream applications such as proteome analysis, biomarker detection, enzyme assays, SELDI analysis, protein array pixelation, 1D and 2D gel electrophoresis, LC/MS, and MALDI-TOF MS.

Magnetic beads (particles) are an entirely different type of solid support matrices from beaded agarose or other porous resins. They are much smaller (typically 1-5 µm diameter), thus providing larger surface areas for a high density of ligand immobilization. The beads are manufactured using nanometer-scale superparamagnetic iron oxide as core and entirely encapsulated by a high purity silica shell, ensuring no leaching problems with the iron oxide. The excellent hydrophilic properties of the beads make them less nonspecific binding.

Workflow of Quick HSA/IgG Depletion Kit

Work with the Albumin Removal Kit is straightforward. Mix the beads with serum or plasma samples and incubate them with continuous rotation. The beads remain suspended in the sample solution during mixing, allowing all other proteins in the sample (except albumin and IgG) to bind to the beads. After incubation, the beads are collected and separated from the sample using a magnet rack. Then the bound proteins are eluted.



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- Remove >95% IgG and 90% Albumin.
- High throughput*:* Rapidly process up to 96 samples in less than 30 minutes.
- Low abundance enrichment is equal to or better than hexapeptides or antibodies
- Mild elution preserves the tertiary structure and allows for easy transfer to secondary analysis.
- Scalable -easily adjusts for sample size and automation
- Minimal sample dilution
- Suitable for LC-MS, activity-based protein profiling, and proteomic research.

Specificities	
Composition	Magnetic beads modified with immobilized with proprietary chemistry
Magnetization	~60 EMU/g
Type of Magnetization	Superparamagnetic
Effective Density	2.5 g/ml
Concentration	30 mg/ml (dH_2O)
Binding Capacity	10µl serum /30µl of Beads
Storage	Store at 4°C upon receipt

Protocol

Note

- The following protocol is an example. Protein concentrations in serum might vary. Ten microliters of human serum have about 700µg of complete protein. About 70% of that will be human serum albumin and IgG. The amount of serum used in each response should be optimized by the user. Overloading the system may result in albumin carryover into the low abundant protein fraction. Using the given protocol, the user should expect a typical elution to include 50-100µg of serum protein.
- The samples are eluted in a salt-containing buffer and may need to be desalted before electrophoresis or mass spectrometry analysis. If the sample is sufficiently diluted before analysis, desalting may not be required.

Materials Required

- BcMag™ Quick HSA-IgG Depletion Magnetic Beads
- 1x Binding/Washing Buffer: 20 mM sodium phosphate, pH 6.5.
- 1x Elution Buffer: 100 mM glycine-HCl, pH 2.7

Equipment

Item	Source
Magnetic rack for centrifuge tube ** Based on sample volume, the user can choose one of the following magnetic Racks	• BcMag rack-2 for holding two individual 1.5 ml centrifuge tubes (Bioclone, Cat. # MS-01) • BcMag rack-6 for holding six individual 1.5 ml centrifuge tubes (Bioclone, Cat. # MS-02) • BcMag rack-24 for holding twenty-four individual 1.5-2.0 ml centrifuge tubes (Bioclone, Cat. # MS-03) • BcMag rack-50 for holding one 50 ml centrifuge tube, one 15 ml centrifuge tube, and four individual 1.5 ml centrifuge tubes (Bioclone, Cat. # MS-04)
BcMag 96-well Plate Magnetic Rack.	• BcMag 96-well Plate Magnetic Rack (side-pull) compatible with 96-well PCR plate and 96-well Microplate or other compatible racks (Blioclone, Cat#: MS-06)
Adjustable Single and Multichannel pipettes	
Centrifuge with swinging bucket	
Addition items are required if using 96-well PCR plates/tubes	
Vortex Mixer ** The user can also use other compatible vortex mixers. However, the time and speed should be optimized, and the mixer should be Orbit ≥1.5 mm-4 mm, Speed ≥ 2000 rpm	
Eppendorf™ MixMate™	Eppendorf, Cat#:5353000529
Tube Holder PCR 96	Eppendorf, Cat#: 022674005
Tube Holder 1.5/2.0 mL, for 24 × 1.5 mL or 2.0 mL	Eppendorf, Cat#: 022674048
Smart Mixer, Multi Shaker	Benchtop Lab Systems, Cat#:5353000529

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1.5/2.0 mL centrifuge tube	
96-well PCR Plates or 8-Strip PCR Tubes	
PCR plates/tubes ** ***IMPORTANT!*** If using other tubes or PCR plates, ensure that the well diameter at the bottom of the conical section of PCR Tubes or PCR plates must be ≥2.5mm.	
Addition items are required if using 96-well microplates	
Fisher Scientific™ Microplate Advanced Vortex Mixers	Fisher, Cat#:02-216-101
OHAUS Microplate Vortex Mixers	OHAUS, Cat#:30392160
Vortex Mixer ** The user can also use other compatible vortex mixers. However, the time and speed should be optimized, and the mixer should be Orbit ≥1.5 mm-4 mm, speed≥ 800 rpm	
Clear Flat-bottom Non-Binding Assay Microplates	

Procedure

Note:

- Vigorously shake the bottle until the magnetic beads become homogeneous.
- Do not allow the magnetic beads to sit for more than 5 minutes before dispensing.

1. Transfer 40 µl beads to a fresh tube or a new 96well PCR plate, Microplate, or 0.2ml PCR tube and add 50 µl binding buffer to a final 90 µL reaction volume.
2. Add 10 µl serum sample and mix the sample with beads by slowly pipetting up and down 20-25 times, or Vortex the sample at 2000 rpm for 5 minutes (see picture).
3. Place the sample plate/ tube on the magnetic separation plate for 30 seconds or until the solution is clear.
4. Discard the supernatant while the sample plate remains on the magnetic separation plate.
5. Wash the beads with 100 µL of the binding buffer by slowly pipetting up and down 20-25 times, or Vortex the sample at 2000 rpm for 2 minutes. Place the sample plate/ tube on the magnetic separation plate for 30 seconds or until the solution is clear.
6. Repeat step 5 once.
7. Resuspend the beads in 50-100µl of Elution Buffer and mix the sample with beads by slowly pipetting up and down 20-25 times, *or* Vortex the sample at 2000 rpm for 5 minutes.
8. Transfer the supernatant to a fresh microcentrifuge tube or microtiter plate well. The supernatant containing the proteins of interest is ready for downstream applications.

Related Products	
Product Name	Product Name
One-Step Lipids Removal Kit	Quick Albumin Removal Kit
One-Step Deproteinizing Kit	Quick HSA and IgG Depletion Kit
One-Step SDS Removal Kit	One-Step Dye Removal Kit
One-Step Detergent Removal Kit	Quick Endotoxin Removal Kit
EDTA Metal Ion removal Kit	Immobilized TCEP Disulfide Reducing Kit
EGTA Metal Ion removal Kit	One-Step PCR Inhibitor Removal Kit
One-Step DNA and RNA Cleanup Kit	One-Step DNA and RNA Removal Kit
One-Step Sequencing Cleanup Kit	One-Step Single-Stranded DNA Removal Kit
One-Step Fluorescent Labeling Cleanup Kit	One-Step RNA Removal Kit
One-Step NGS Cleanup Kit	One-Step PCR Cleanup Kit

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