Related items available from ADI

Catalog# ProdDescription

700-1250-10 Albumin-X, Albumin (multiple species) removal kit (sufficient to remove 6-10 mg albumin or process ~200-300 ul serum; **10 mini-columns** ~1.25 ml resin)

800-200-BSA Albumin (Human, Mouse, rat, bovine and others) removal kit (synthetic dye based matrix; sufficient to remove 20-40 mg BSA from Bioprocessed material), **2 ml** aff column

800-205-BSA Albumin (Human, Mouse, rat, bovine and others) removal kit (synthetic dye based matrix; sufficient to remove 50-100 mg BSA from Bioprocessed material), **5 ml** aff column

800-225-BSA Albumin (Human, Mouse, rat, bovine and others) removal kit (synthetic dye based matrix; sufficient to remove 250-500 mg BSA from Bioprocessed material), **25 ml** aff column

8000 Bovine Albumin (BSA) ELISA Kit, 96 tests, Quantitative

800-110-PRA Protein-A ELISA Kit, 96 tests, Quantitative

800-120-PRG Protein-G ELISA Kit, 96 tests, Quantitative

800-130-ECP E Coli proteins (5 strains) host cell proteins (HCPs) ELISA kit, 96 tests

800-140-CHO Chinese Hamster Ovary Cell (CHO) host cell Proteins (HCPs) ELISA kit, 96 tests

Albumin+IgG and Transferrin removal kits also available.

#1200 Human Serum Albumin ELISA kit
#6300 Mouse Serum Albumin ELISA kit
#6400 Rat Serum Albumin ELISA kit
#8000 Bovine Serum Albumin ELISA kit

Albumin-X ™ Albumin (multiple species) removal kit

Cat. # 800-200-BSA (2 ml)
Cat. # 800-205-BSA (5 ml)
Cat. # 800-225-BSA (25 ml)

Albumin (Human, Mouse, rat, bovine and others) removal kit (synthetic dye based matrix; sufficient to remove 20-40 mg BSA from Bio-processed material)

For In Vitro Research Use Only

ALPHA DIAGNOSTIC INTERNATIONAL

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Albumin-X ™ Albumin (multiple species) removal kit # 800-200-225 & 700-1250-10

Kit Contents: (Sufficient reagents for 10 serum or plasma samples)

Kit Components	Cat#
Albumin-X, albumin removal columns, 1 ready to use **column ~2 ml # C-800200** .	1 column in # 800-200-BSA
Albumin-X, albumin removal columns, 1 ready to use **column ~5 ml # C-800205** .	1 column in # 800-205-BSA
Albumin-X, albumin removal columns, 10ready to use **column ~25 ml # C-800225** .	1 column in # 800-225-BSA
Albumin **Binding buffer**, pH 7.1, 50 ml dilute with use #A125012	1 bottle
Albumin **elution buffer** pH 8.0, ready to use, 50 ml #A125013	1 bottle
Complete Instruction Manual	800-200-BSA
Store kit at 4oC. **Albumin binding resin must never be frozen**.	
Note: Three kits are essentially the same except the column size and share binding and elution buffers.	

INTRODUCTION

Serum or plasma is a very complex and dynamically changing pool of proteins that are produced and secreted by many different tissues and organs into the blood. Therefore, blood contains 1000s of proteins varying in concentrations from several mg/ml to pg/ml. Serum protein analyses has been used to monitor the presence or absence of proteins monitor a given disease or physiological state of an organism. However, complexity of serum proteins and high concentrations offers a challenge to identify low abundance proteins. Serum albumin alone may constitute up to 50% of total protein mass. Other high abundance proteins are Ig's, Transferrin (Tf), hemoglobin (Hb), alpha 2 macroglobuin, fibrinogen, C-reactive proteins etc. These high abundance proteins makes it difficult to see the low abundance proteins during electrophoresis (single or 2-dimension) or isoelectric focusing and other analytical techniques. Therefore, removal of albumin alone or in combination with other proteins, greatly enhances the separation or resolution of low abundance proteins by common analytical techniques.

ADI's Albumin removal kit provides a convenient, specific, and highly efficient method for removing albumin from serum, plasma or other biological samples. Albumin removal columns contains a high affinity albumin binding resin packed in ready-to-use mini columns. The kit also contains albumin binding buffers and elution buffers. The kit contains 10 columns (resin volume ~1.25 volume) to

QUALITY CONTROL

It is recommended that the users optimize the sample volume or proteins concn for a given sample and the Albumin-X removal column capacity. Overloading or exceeding the column capacity will result in the unbound fractions showing higher concentrations of albumin. Sample volume protein concentration can be determined using BCA protein assay before and after adsorption on the column. Samples can also be analyzed by SDS-PAGE or 2-D SDS-PAGE, Western blot or ELISA.

ADI also has albumin quantitation ELISA kits for Mouse, rat, and human that can be used for quality control as well.

#1200 Human Serum Albumin ELISA kit
#6300 Mouse Serum Albumin ELISA kit
#6400 Rat Serum Albumin ELISA kit
#8000 Bovine Serum Albumin ELISA kit

Antibodies to various species albumins are also available. Please contact ADI for specific requirements.

Limitations of the Albumin-X removal kit

1. This kit has been optimized with human serum. Other species serum or plasma sample should be optimized for optimal binding.

2. Sample buffer should be compatible with the kti or the sample should be dialyzed with the binding buffer before us.

3. Albumin binding dye may bind proteins other than albumin. Its usage must be tested to make sure that protein under study is not lost or bind to the column. ADI has antibody based albumin removal kits that will remove only the albumin from samples. Please contact ADI for details.

4. This kit is designed to be used for a single use to avoid sample contamination.

5. Albumin-X binding column must not be run dry or frozen.

Store all reagents at 2-8oC. Albumin removal columns must never be frozen. All kit components are stable for at least 12 months from the date of manufacture under proper storage and usage conditions.

Technical Protocol for removing albumin *(ALLOW ALL REAGENTS TO REACH ROOM TEMPERATURE 22-28oC BEFORE USE).* Remove required # of albumin removal columns, label and arrange in an appropriate tube rack. .

1. Break-off the lower tip of column and allow storage buffer to drain by gravity flow or put the column in 1.5-2 ml or 15-ml collection tube and centrifuge (30 seconds at 3000 rpm). Discard the buffer and apply 1-2 ml of the 1X binding buffer and let it run through the column. When buffer stops flowing then close the bottom with the supplied end-caps and top caps.

2. If processing samples that contains unknown buffers or high salts or pH, it is recommended to dialyze the samples for several hours or overnight at 2-4oC in at least 10-20 times the samples volume in 1X binding buffer or in 20mM phosphate buffer, pH 7.1 (not supplied in the kit). High salt concn or unusual pH will decrease the albumin binding.

3. When processing samples containing high concn of albumin (serum or plasma etc), it is important not to exceed the column biding capacity. Serum or plasma, due to high concn of proteins are quite dense or viscous, it would help if samples can be diluted at least 1:1 or more (1:5-1:10) with albumin binding buffer to reduce sample viscosity and allow more efficient binding to the column. If sample dilution is undesirable, then it is possible to load the sample directly on the column.

4. Centrifuge or drain the column as in step 1 to remove the binding buffer and immediately apply the samples and let it enter the column and close the column with the end-cap. Allow the sample to incubate for 30 minutes at room temperature. If sample volume is >0.5 ml then allow the samples to pass through the column using gravity flow and re-apply the eluate 2-3 times for more efficient removal of albumin.

5. For small sample volume (<0.5 mls), centrifuge the column once and collect the unbound fraction that will be depleted of albumin. For high volume samples, collect the unbound fraction by gravity

6. flow and then collect the remaining by centrifugation and combine the fractions. Albumin depleted samples can then be used or stored at -20oC for downstream processing.

7. If it is necessary to analyze the bound proteins, then wash the column with 10-volumes of binding buffer (or PBS) until the A280 of the eluate is <0.1. Elute the bound proteins with loading 5-ml of the supplied elution buffer and collecting the eluate by gravity flow.
8. Re-equilibrate the column with by passing 3-5 mls of the binding buffer, close the column with end-caps and top caps. Store at 2-4oC.
9. Albumin-X removal column are designed for single use only to avoid contamination. If sample contamination is not the issue then it is possible to re-use the columns 5-10 times.

10. Re-equilibrate the column with by passing 1X binding buffer (3X the volume of the column size). After equilibration with the buffer, close the column with end-caps and top caps. Store at 2-4oC.

11. Albumin-X removal column are designed for single use only to avoid contamination. If sample contamination is not the issue then it is possible to re-use the columns 5-10 times.

Albumin-X removal column-Technical Notes

Albumin binding Dye content: ~2 mg/ml of beads
Particle size: 100-200 mesh
Purified human serum albumin binding capacity ~10 mg/ml
Human serum capacity ~0.2-03 ml serum per ml of gel (serum or plasma capacity from other species may vary).
Shipping buffer: PBS, pH 7.4 and 0.1% azide
Stability ~1 year at 4oC in the supplied buffer
Stability pH range ~4-10

Note: Serum albumin concn is ~20-40 mg/ml 9av 30 mg/ml) in most species or 3 mg/100 ul. In general, serum or plasma volume to albumin binding resin in the column is ~1:3 (v/v; eg., 100 ul serum to 300 ul resin).

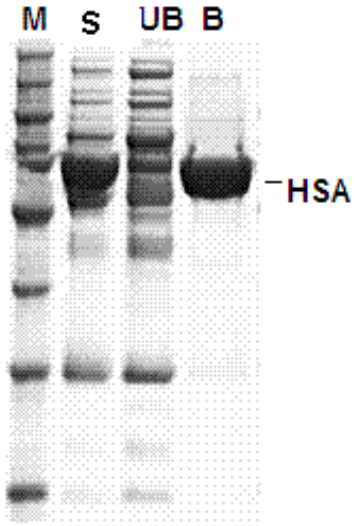
Species Specificity

The dye used in the column is known to binding albumin from many species (human, monkey, mouse, rat, G. pigs, rabbits, etc). Albumin biding dye also However albumin from various species bind to the albumin column at various species. Following species albumin have been tested to bind the Albumin-X removal column

Species	Relative binding**
Human	100%
Rabbit	>80%
Rat	>80%
Mouse	>70%
Pig	>70%
Bovine	>40%

**actual binding may vary depending the method of albumin purification, proteins conc, buffer composition, pH, and flow rate.

Albumin binding dye can also bind many other proteins (Kinases, dehydrogenases, and other nucleotide-dependent proteins and enzymes) to a varying degree.



Removal of albumin from human serum using Albumin-X removal column: Human serum (0.2 ml) was processed on Albumin-X removal column and 20 ug protein samples were resolved on SDS-APGE followed by coomassie staining. M=Mol Wt markers; **S**=Serum; **UB**=unbound; B=Bound or eluted proteins. The column removed >90% of the serum albumin without binding to too many other proteins.

process and remove majority of albumin from up to 250-300 uls of serum or plasma. Samples containing less concentration of albumin (such as culture media, amniotic fluid or cells/tissue extracts) can also be processed. The processed samples are suitable for downstream processing such as SDS-PAGE (1 or 2-D) and mass specs or toxins or IgG binding assays.

PRINCIPLE OF THE TEST

Albumin-X removal kits contains a modified synthetic blue dye that has high affinity for albumin and other proteins and enzymes. Samples containing albumin (diluted with the supplied albumin binding buffer or undiluted) are passed over the albumin binding resin and the unbound fractions (albumin depleted) is collected. Bound proteins (albumin and others) can be eluted if necessary with the supplied elution buffer.

MATERIALS AND EQUIPMENT REQUIRED

Adjustable micropipet (25-100 μl) and multichannel pipet with disposable plastic tips. Reagent troughs, plate washer (recommended) and ELISA plate Reader.

PRECAUTIONS

The Alpha Diagnostic International Albumin-X removal kit is intended for *in vitro research* use only. .Applicable **MSDS**, if not already on file, for 0.005% azide in Kit components (albumin removal resins, binding and elution buffers contains 0.1% (v/v) Proclin-300. obtained from ADI or the web site.

SPECIMEN COLLECTION AND HANDLING

Recommended samples are serum or plasma or culture media containing albumin. Other biological fluids such amniotic fluids cells and tissue extracts can be used but their usage must be optimized.

For serum collect whole blood without anticoagulant and allow blood to clot between 2-8°. Serum should be promptly separated, preferably in a refrigerated centrifuge, and stored at -20oC or lower. For plasma, collect blood in heparinized or EDTA-tubes, collect plasma stored samples at -20oC or below. Do not store samples in self-defrosting freezers. Avoid repeated freezing and thawing of samples. For long term storage of samples, it is recommended that samples should be aliquoted into sample tubes or vials prior to freezing. Prior to use, allow all specimens to come to room temperature (22oC to 28oC) and mix by gentle inversion or swirling. Samples should be centrifuged or filtered 0.45 u membrane to remove any particulate material that may clog the albumin binding column and disrupt the flow of samples.