

## Related items available from ADI

Catalog#	ProdDescription
<b>#800-200-225</b>	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)
<b>#800-200-225</b>	Albumin-X, Albumin (multiple species) removal kit (sufficient to remove 2-3 mg albumin or process ~50-100 ul serum; 10 mini-columns ~250 ul resin)
<b>#800-325-BSA</b>	Bovine serum albumin (BSA) removal kit (Antibody based aff matrix; sufficient to remove 25-50 mg BSA from Bioprocessed material), 25 ml aff column
<b>#800-310-BSA</b>	Bovine serum albumin (BSA) removal kit (Antibody based aff matrix; sufficient to remove 10-20 mg BSA from Bioprocessed material), 10 ml aff column
<b>#800-302-BSA</b>	Bovine serum albumin (BSA) removal kit (Antibody based aff matrix; sufficient to remove 1-5 mg BSA from Bioprocessed material), 2 ml aff column
<b>#800-225-BSA</b>	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
<b>#800-205-BSA</b>	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
<b>#800-200-BSA</b>	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

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

Instruction Manual No. M-800-332-AGT

## Bovine Serum Albumin, IgG, and Transferrin BSA Removal kit

**Cat. # 800-332-AGT**

**BSA, IgG, and Transferrin removal kit from Bio-processed material (Antibody based matrix & re-usable)**

*For In Vitro Research Use Only*



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## Bovine Serum Albumin, IgG, and Transferrin removal Kit kit # 800-332-AGT: Kit Contents:

Kit Components	Cat #
Rabbit Anti-BSA IgG, Goat Anti-IgG, and rabbit anti-transferrin IgG agarose column, 2 ml settled gel in the column # 800332C.	1 column in # 800-332-AGT
Other reagents supplied with all of the above kits	
Protein Binding/regeneration buffer, (20X); 25 ml, dilute 1:20 with water #800302-BB	1 bottle
Protein elution buffer (10X), 10 ml, #800302-EB	1 bottle
Complete Instruction Manual	800-332-AGT
Store kit at 4oC. Albumin binding resin must never be frozen.	
<b>Note:</b> All kits are essentially the same except the column size and share binding and elution buffers.	

BSA (bovine serum albumin) is often used as a carrier protein to couple haptens (peptides, drug, steroid etc) for the purpose of making antibodies. Antibodies to BSA can be used to improve the functioning of the antibodies in a given immunoassay. Bovine serum or fetal bovine serum (contains BSA, IgG, Transferrin and other serum proteins) is also used in culture medium or as an additive in purified protein or other biological material. Often the bovine additives should be removed for various analyses such as western blot or ELISA or mass spectrum analyses. Antibodies to BSA, IgG and transferrin can be used to prepare an affinity column to deplete or remove these proteins from samples. We have used highly specific antibodies and coupled to CNBR-activated Agarose. The affinity matrix contains ~2-4 mg each of Rabbit anti-BSA IgG, Rabbit anti-bovine IgG, and goat anti-transferrin IgG (mix) per ml of Agarose (affinity column).

The affinity column can be used to deplete or remove bovine albumin, IgG, and transferrin from various samples. Like all affinity column, the binding capacity for this affinity column may be different depending upon the concentration of each protein in the sample and also the complexity of the sample (bovine serum or purified protein additives).

ADI's BSA removal kit provides a convenient, specific, and highly efficient method for removing (BSA) from serum, plasma or other biological samples. BSA removal columns contains a high affinity albumin binding resin packed in ready-to-use mini columns. The kit also contains BSA binding buffers, and regeneration buffers. The column can be used at least 5-10 times depending upon the sample and usage.

Samples containing low 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.

### Limitations of the BSA, IgG, and Transferrin depletion/removal kit

1. Antibodies used in the kit are specific for albumin, IgG, and transferrin from bovine and show minimal reactivity with human, mouse or rat albumin. This kit is not recommended to remove these proteins from species other than bovine.
2. Binding capacity of the affinity matrix for BSA, IgG, and Transferrin may vary depending upon the complexity of the sample (serum, culture medium or purified proteins). The binding of the proteins may also independently vary depending upon the sample. For example, BSA may bind well, whereas transferrin or IgG may not.
3. Sample buffer should be compatible with the kit or the sample should be dialyzed with the binding buffer before use.
4. Overloading of samples may give the impression that the affinity column is not functioning. Therefore, we recommend testing various amounts of samples to test the binding capacity for each protein (BSA, IgG, and Transferrin).
5. This kit can be re-used after elution of BSA and re-used 5-10 time if column is processed and stored properly.
6. BSA-X binding column must not be allowed to run dry or frozen

**Store all reagents at 2-8oC.** BSA 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*). The affinity column contains ~2 ml of settle gel containing covalently linked anti-BSA, Anti-IgG, and anti-Tf IgG. It is possible to use this as a single column or the affinity matrix subdivided into 0.5-1 ml size to process multiple samples. Dilute binding and elution buffers with water in required volume. Diluted buffers can be stored at 4oC for 1-2 week.

1. Break-off the lower tip of column and allow storage buffer to drain by gravity flow. Discard the buffer and apply 1-2 volumes 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 PBS pH 7.4 (not supplied) with at least 10-20 times the samples volume. Culture media, serum or plasma should be dilution at least 1:2-1:10 with the binding buffer before loading on the column. High salt concn or unusual pH will decrease the protein binding.
3. When processing samples containing high concentrations of albumin, IgG and Tf (serum or plasma etc), it is important not to exceed the column binding 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 the 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. 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. It is recommended to re-load the eluate over the affinity column 2-3 times for more efficient binding of proteins.
5. For high volume samples, collect the unbound fraction by gravity flow. Protein depleted samples can then be used or stored at -20oC for downstream processing

6. If it is necessary to analyze the bound protein (Albumin, BSA, and Tf), then wash the column with 5-volumes of binding buffer (or PBS) until the A280 of the eluate is <0.1. Elute the bound proteins with 3-column volume of the elution buffer (e.g., for 2 ml column, use at least 6-8 ml elution buffer) of the supplied elution buffer and collecting the eluate by gravity flow. It is recommended to elute proteins in appropriate size fractions (e.g. 0.2-0.5 mls) and analyze protein concn by A280 to determine protein containing fractions. Pool appropriate fractions and immediately neutralize it with 1/10<sup>th</sup> volume of 1 M Tris, pH 8.0 (not supplied in kit) . Eluted proteins can be dialyzed against appropriate buffer (e.g., PBS, pH 7.4) and analyzed.
7. Re-equilibrate the column with by passing 3-5 column volumes of the binding buffer, close the column with end-caps and top caps. Store at 2-4oC.
8. Affinity 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.
9. 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.

### **BSA, IgG, and Transferrin depletion/removal column-Technical Notes**

**Bovine Albumin, IgG, and Transferrin binding antibody content:** ~1-2 mg/ml of beads

**Stability** ~1 year at 4oC in the supplied buffer

**Note:** Serum albumin concn is ~20-40 mg/ml (av 30 mg/ml) in most species or 3 mg/100 ul. A preliminary determination of the column size and samples size must be made based upon the sample and BSA concentration.

### **Species Specificity**

The antibodies used in this kit have been shown by immunoelectrophoresis and ELISA to react specifically with bovine, and to have essentially no reactivity with other bovine serum proteins..

Serum from the following species, assayed at a 1% serum concentration, showed less than 5 ng/ml reactivity in the assay: human, monkey, sheep, goat, rabbit, hamster, mouse

The antibodies used in the kit are specific for bovine serum albumin, IgG, and Tf with minimal reactivity with mouse, rat, and human and other species.

Please contact ADI if you need to remove albumin from mouse, rat, or human samples.

## QUALITY CONTROL

It is recommended that the users optimize the sample volume or proteins concn for a given sample for use with the affinity column. Overloading or exceeding the column capacity will result in the unbound fractions showing higher concentrations of BSA or IgG or Tf. Sample volume protein concentration can be determined using A280 or 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.

BSA ELISA kit #8100  
Bovine IgG ELISA Kit #8010  
Bovine Tf (Transferrin) ELISA kit #8070

### #8000 Bovine Serum Albumin ELISA kit

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

AntibodyType	Catalog#	ProdDescription
Rabbit-Poly (BSA) protein IgG	ALBB14-A	Anti-Bovine Serum Albumin
Rabbit-Poly	80519 & 80517	Anti-Bovine IgG
Rabbit-Poly	Tf19-A	Anti-Bovine Transferrin IgG

## PRINCIPLE OF THE TEST

Bovine Albumin, IgG, and Tf removal kits contains a high titer antibodies specific for bovine serum albumin (BSA), IgG, and Tf coupled to agarose. Samples containing BSA, IgG, and Tf (diluted with the supplied albumin binding buffer or undiluted) are passed over the antibody-affinity column and the unbound fractions (albumin, IgG, and Tf depleted) are collected. Bound proteins (albumin, IgG, and Tf) can be eluted to regenerated the affinity column for re-use.

## MATERIALS AND EQUIPMENT REQUIRED

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

## PRECAUTIONS

The Alpha Diagnostic International affinity removal kit is intended for *in vitro research* use only. Applicable **MSDS**, if not already on file, for Proclin-300 (0.1% v/v) in Kit components (affinity column), can be obtained from ADI or the web site.

## SPECIMEN COLLECTION AND HANDLING

Recommended samples are diluted serum or plasma or culture media containing BSA, IgG and Tf. 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.