ELISA Kit Components	Amount	Part No.
Anti-Bovine Albumin Microwell Plate	8-well strips (12)	8101
Bovine Albumin Control	0.65 ml	8102
Bovine Albumin Standard 1 ng/ml	0.65 ml	8103B
Bovine Albumin Standard 5ng/ml	0.65 ml	8103C
Bovine Albumin Standard 20ng/ml	0.65 ml	8103D
Bovine Albumin Standard 40ng/ml	0.65 ml	8103E
Bovine Albumin Standard 80 ng/ml	0.65 ml	8103F
Anti-BSA HRP Conjugate (100X)	0.15 ml	8104
HRP Conjugate Diluent	12 ml	S-046
Sample Diluent Concentrate (20X)	10 ml	SD-20B
Wash Solution Concentrate (100X)	10 ml	WB-100
TMB Substrate	12 ml	80091
Stop Solution	12 ml	80101
Product Manual	1 ea	M-8100

Notes

For more details please consult our web site (www.4adi.com) or contact us by email (service@4adi.com).

Instruction Manual No. M-8100

Bovine Serum Albumin (BSA) ELISA Kit

Cat. No. 8100, 96 tests

For Detection and Quantitation of Residual
Bovine Albumin (BSA) in Biological Solutions

For research use only, not for diagnostic or therapeutic use

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INTENDED USE

The Bovine Albumin ELISA Kit is a sandwich immunoassay for the quantification of bovine serum albumin (BSA) in proteins or antibody samples, recombinant proteins, cell extracts, cultured cells, culture media or any other biological samples that were exposed to bovine serum or fetal bovine serum and may contain traces of BSA (host cell protein or HCPs). Due to high sensitivity of the kit and the high concentration of albumin in serum, this kit may not be suitable for measuring albumin in serum, or in situations where BSA may be prevalent as a contaminant in the testing environment. The ADI ELISA kit # 8000 is recommended as an alternative for measuring BSA in serum or other samples containing high BSA concentrations. This kit is for research use only.

INTRODUCTION

Albumin is the protein of the highest concentration in plasma. Albumin transports many small molecules in the blood (for example, bilirubin, calcium, progesterone, and drugs). It is also of prime importance in maintaining the osmotic pressure of the blood. Albumin is synthesized by the liver. Albumin performs many functions including maintaining the "osmotic pressure" that causes fluid to remain within the blood stream instead of leaking out into the tissues. Liver disease, kidney disease, and malnutrition are the major causes of low albumin.

BSA or albumin is widely used as a carrier protein in preparations of purified proteins, recombinant proteins or cell extracts. Fetal calf serum (FCS) or fetal bovine serum (FBS) is also used for the maintenance of cultured cells or cell lines. Many important products, such as recombinant protein or cells, are then used for human therapeutics. This can result in residual contamination of the desired product by components (BSA and other serum proteins) used in the culture media. The use of “serum free” or defined media greatly reduces the serum dependence. Most commercial formulations of serum free media contain significant amounts of albumin and transferrin either of bovine or human origin, and insulin from various species. Therefore, purified products may contain albumin or other components that may create potential health risks or other problems that might result from trace contaminants. A great effort is required to reduce trace media contamination to the lowest levels practical through optimal process design and validation. Final product testing for these contaminants requires a highly sensitive and reliable method.

The ADI BSA ELISA kit is a simple, easy to use, and highly sensitive method to detect BSA contamination to less than <500 pg/mL. This kit can be used to measure BSA as routine quality control of in-process streams as well as final product.

PRINCIPLE OF THE TEST

The Bovine Albumin ELISA kit is based on the binding of bovine albumin in samples to two antibodies, one immobilized on the microtiter wells, and the other conjugated to horseradish peroxidase (HRP). After a washing step, chromogenic substrate is added and color is developed by the enzymatic reaction of HRP on the TMB substrate, which is directly proportional to the amount of BSA present in the sample. Stopping Solution is added to terminate the reaction, and absorbance at 450nm is then measured using an ELISA microtiter well reader. The concentration of BSA in samples is calculated from a standard curve of purified bovine albumin of designated concentration.

STORAGE AND STABILITY

The microtiter well plate and all other reagents, if unopened, are stable at 2-8^{O}C until the expiration date printed on the box label. Stabilities of the working solutions are indicated under Reagent Preparation.

PERFORMANCE CHARACTERISTICS &EXPECTED RESULTS

Specificity

The antibodies used in this kit have been shown by immunoelectrophoresis and ELISA to react specifically with BSA, 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.

Value-assignment of Standards

The concentrations of standards in the kit have been calibrated to BSA standard preparations from Sigma Chemical, cat# P0914, and from Thermo Scientific, cat#23209, a secondary reference to the NIST BSA #927e.

Sensitivity

The 3 SD range of a 0.5ng/ml sample was shown not to overlap the 3 SD range of the negative sample diluent, based on the assay of 13 replicates in 3 runs. Therefore, the Bovine Albumin ELISA can be considered to have a sensitivity of **250-500 pg/ml**.

Normal Range

Assay of fetal bovine serum from four (4) different sources showed an albumin concentration range of 6.9 to 15.2 mg/ml.

Precision

Samples containing low and medium concentrations of BSA were assayed as duplicates in multiple assays (n=5) to obtain between-assay reproducibility. Coefficients of variation (CVs) were calculated for the concentrations using a point-to-point curve-fitting program.

BSA concentrations were measured with good between-assay (5.7 to 9.7 %CV) reproducibility.

Sample	BSA Concn	Inter-assay %CV
Low Concentration	2.5 ng/ml	**9.7**
Medium Concentration	10 ng/ml	**5.7**

CALCULATION OF RESULTS

The results may be calculated using any immunoassay software package. The four-parameter curve-fit is recommended. If software is not available, Bovine Albumin concentrations may be determined as follows:

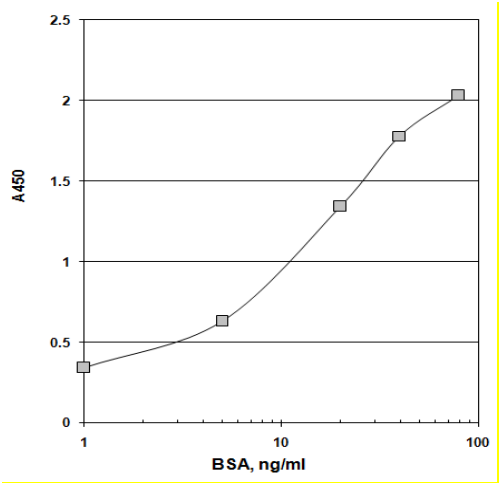
1. Calculate the mean OD of duplicate samples.
2. On graph paper plot the mean OD of the standards (y-axis) against the concentration (ng/ml) of Bovine Albumin (x-axis). Draw the best fit curve through these points to construct the standard curve. A point-to-point construction is most common and reliable.
3. The Bovine Albumin concentrations in unknown samples and controls can be determined by interpolation from the standard curve.
4. Multiply the values obtained for the samples by the dilution factor of each sample.
5. Samples producing signals higher than the 80 ng/ml standard should be further diluted and re-assayed.

TYPICAL RESULTS

The following data are for illustration purposes only. A complete standard curve should be run in every assay to determine sample values.

Wells	Standards, Control & Samples	A450 nm mean	BSA ng/ml
1A, B	**Negative Diluent Control**	0.12	0
1C, D	1ng/ml **Standard**	0.34	1
1E, F	5ng/ml **Standard**	0.63	5
1G, H	20ng/ml **Standard**	1.34	20
2A, B	40ng/ml **Standard**	1.78	40
2C, D	80ng/ml **Standard**	2.03	80
2E, F	**Positive Serum Control** [Value: 7 – 13ng/ml]	0.92	11
2G, H	**Sample**	0.21	<1

A typical assay Standard Curve (do not use for calculating sample values)



KIT CONTENTS

Ready For Use: Store as indicated on labels.

Component	Part #	Amt	Contents
Anti-Bovine Albumin Microwell Strip Plate	8101	8-well strips (12)	Coated with purified anti-bovine albumin antibodies. Return unused strips to the pouch with desiccant; re-seal and store refrigerated.
Positive Control [BSA] range on label	8102	0.65 ml	BSA with stated concentration range; diluted in buffer with detergents and antimicrobial as stabilizers.
Bovine Albumin Standards			
1 ng/ml 5 ng/ml 20 ng/ml 40 ng/ml 80 ng/ml	8103B 8103C 8103D 8103E 8103F	0.65 ml 0.65 ml 0.65 ml 0.65 ml 0.65 ml	Five (5) vials, each containing the specified concentration of BSA; diluted in buffer with detergents and antimicrobial as stabilizers.
BSA Standards are calibrated to secondary references of NIST #927e.			
TMB Substrate	80091	12 ml	Chromogenic substrate for HRP containing TMB and peroxide.
Stop Solution	80101	12 ml	Diluted sulfuric acid.

To Be Reconstituted: Store as indicated.

Component	Instructions for Use
Sample Diluent Concentrate (20x) Cat. No. SD-20B, 10ml	Dilute the entire volume, 10ml + 190ml with distilled or deionized water into a clean stock bottle. Label as **Working Sample Diluent** and store at 2-8ºC until the kit lot expires or is used up.
Wash Solution Concentrate (100x) Cat. No. WB-100, 10ml	Dilute the entire volume, 10ml, to 1L with distilled or deionized water into a clean stock bottle. Label as **Working Wash Solution** and store at ambient temperature until kit is used entirely.
Anti-BSA-HRP Conjugate Concentrate (100x) Part No. 8104, 0.15ml	Peroxidase conjugated anti-bovine albumin antibody in buffer with detergents and antimicrobial as stabilizers. Dilute fresh as needed; 10ul of concentrate to 1ml of **HRP Conjugate Diluent** is sufficient for 1 8-well strip. Prepare 10 ml for a full plate. Use within the working day and discard. Return concentrate to 2-8ºC storage.
HRP Conjugate Diluent Part No. S-046, 12 ml *Use As-Is*	

Materials Required and Not Provided:

- Pipettors and pipettes that deliver 100ul and 1-10ml. A multi-channel pipettor is recommended.
- Disposable glass or plastic 5-15ml tubes for diluting samples and Anti-BSA-HRP Concentrate.
- Graduated cylinder to dilute Wash Concentrate and Sample Diluent concentrate; 200ml to 1L, Stock bottles. **Vessels must NEVER have been used to measure BSA-containing reagents.**
- Distilled or deionized water to dilute reagent concentrates.

PRECAUTIONS AND SAFETY INSTRUCTIONS

Standards, Controls, Sample Diluent, and HRP Antibody contain bromonitrodioxane (BND: 0.05%, w/v). Stop Solution contains 1% sulfuric acid. Follow good laboratory practices, and avoid ingestion or contact of any reagent with skin, eyes or mucous membranes. All reagents may be disposed of down a drain with copious amounts of water.

MSDS for TMB, sulfuric acid and BND, if not already on file, can be requested or obtained from the ADI website.

SPECIMEN COLLECTION AND HANDLING

Culture media, cell extracts, recombinant proteins or other biological fluids may be used as samples with proper dilution to avoid solution matrix interference. Effects of chaotropic agents (urea, guanidine-HCl, etc), detergents (NP40, SDS etc), high salt concentrations, or low or high pH have not been studied. Samples containing these reagents should be diluted in Working Sample Diluent to avoid assay interference. The high BSA Standard can be mixed with the diluted sample(s) to assess proper BSA recovery.

QUALITY CONTROL

Negative controls or blanks. BSA is widely used in laboratory reagents (blocking agents, stabilizing agents, carrier proteins etc) and often contaminates common use vessels and instruments such as beakers, graduated cylinders and ELISA washers. THIS KIT HAS A SENSITIVITY OF < 250-500 pg/ml. It is extremely important to use dedicated, previously unused pipettes, reservoirs, beakers, etc, that will be used for making and storing stock solutions, and for running the assay, in order to assure avoidance of background issues. High blank values (A450=>0.400), poor precision, and other unexpected results may indicate BSA contamination problems. This is not a problem with the kit, and requires that the operator take extra steps to eliminate BSA from the testing environment.

Sample Controls. A Positive Serum Control is provided with the kit, assigned with a BSA concentration value range. Recovery in this range is an indicator of proper assay performance. Each lab should also assay internal control samples, which represent the lab's expected sample population and that are maintained stabilized. A Negative Diluent Control should also be run.

ASSAY PROCEDURE

Bring all reagents to room temperature (18-30° C) equilibration (at least 30 minutes).

DILUTE Samples in 1X Working Sample Diluent. Dilution in other buffers may impact the sample quantification in this assay. For accuracy with samples of high concentration, a multi-level dilution scheme is recommended

For reference standard (Sigma cat# P0914, concn =1 mg/ml), we recommend the following scheme.

1) Original stock 1 mg/ml Std: 0.1 ml + 4.9 ml diluent, mix [Concn = 20 ug/ml BSA],
2) From 20 ug/ml BSA: 0.1 ml + 4.9 ml diluent, mix [Concn = 400 ng/ml BSA],
3) From 400 ng/ml BSA: 0.1 ml + 0.9 ml diluent, mix = **40 ng/ml**

ALL STEPS ARE PERFORMED AT ROOM TEMPERATURE. After each reagent addition, gently tap the plate to mix the well contents prior to beginning incubation.

1. Set-up

- Determine the number of wells for the assay run. Duplicates are recommended to include 10 Standard wells and 2 wells for each sample and control to be assayed.
- Remove the appropriate number of microwell strips from the pouch and return unused strips to the pouch. Reseal the pouch and store refrigerated.
- Add 200-300ul Working Wash Solution to each well and let stand about 5 minutes before sample addition.
- Aspirate or dump the liquid and pat the plate dry on a paper towel.

2. 1st Incubation [100ul –60min; 4 washes]

- Add 100ul of standards, samples and controls each to pre-determined wells.
- Tap the plate gently to mix reagents and incubate for 60 minutes.
- Wash wells 4 times and pat dry on fresh paper towels.

3. 2nd Incubation [100ul – 60min; 5 washes]

- Add 100ul of Working Anti-BSA-HRP Conjugate to each well.
- Incubate for 60 minutes.
- Wash wells 5 times as in step 2.

4. Substrate Incubation [100ul – 15min]

- Add 100ul TMB Substrate to each well. The liquid in positive wells will begin to turn blue.
- Incubate for 15 minutes in the dark, e.g., place in a drawer or closet.
  Note: If your microplate reader does not register optical density (OD) above 2.0, incubate for less time, assuring the top standard does not surpass 2 OD.

5. Stop Step [Stop: 100ul]

- Add 100ul of Stop Solution to each well.
- Tap gently to mix. The enzyme reaction will stop; liquid in the wells will turn yellow.

6. Absorbance Reading

- Use any commercially available microplate reader capable of reading at 450nm wavelength. Use a program suitable for obtaining OD readings, and data calculations if available.
- Read absorbance of the entire plate at 450nm using a single wavelength within 30 minutes after Stop Solution addition. If available, program to subtract OD at 630nm to normalize well background.