ELISA Kit Components	Amount	Cat/Part No.
Bovine Albumin Microwell Plate	8-well strips (12)	8001
Bovine Albumin Standard 0.15 ug/ml	0.25 ml	8003A
Bovine Albumin Standard 0.4 ug/ml	0.25 ml	8003B
Bovine Albumin Standard 1.0 ug/ml	0.25 ml	8003C
Bovine Albumin Standard 2.5 ug/ml	0.25 ml	8003D
Bovine Albumin Standard 6.5 ug/ml	0.25 ml	8003E
Bovine Albumin Standard 15 ug/ml	0.25 ml	8003F
Bovine Albumin Standard 40 ug/ml	0.25 ml	8103G
Anti-BSA HRP Conjugate (100X)	0.15 ml	8004
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-8000

Other ELISA kits available from ADI

Mouse: Albumin, IgA, IgG, IgG1, IgG2a, IgG3, IgG2b, IgM, Leptin, Acrp30, CRP, Haptoglobin, TNF-alpha, VEGF, SAP.

Human: BD-1, BD-2, BD-3, NP-1 **and**: Adiponectin (Acrp30 and gAcrp30), Albumin, Aldosterone, AFP, Angiogenin, Angiopoietin-2, beta-2M, C-peptide, CRP, Cox-2, Ferritin, PSA, fPSA, GH, IgG, IgM, IgA, Insulin, NSE, CA125, CA199, CA242, PAP, SHBG, LH, FSH, TSH, T3, T4, and Steroid ELISA kits (cortisol, E2, testosterone, progesterone etc).

Rat: Albumin, CRP, IgG, IgM, Alpha 1 Acid glycoprotein

Bovine: Albumin, IgG, IgM, Lactoferrin, Transferrin

Monkey: IgM, IgG, IgA, CRP, IgE
Chicken: IgY(G), IgM, Ovalbumin
Rabbit: CRP, IgG
Pig: Albumin, IgG, IgM

Dog: CRP, IgG, IgM
Cat: IgG, IgM
Goat: IgG

For more details please contact email (customerservice@atzlabs.com).

Instruction Manual No. M-8000

Bovine Albumin

ELISA Kit Cat. No. 8000

For Quantitative Determination and of Bovine Serum Albumin (BSA) in Fluids

ALPHA DIAGNOSTIC INTERNATIONAL

India Contact:
Life Technologies (India) Pvt. Ltd.
306, Aggarwal City Mall, Opposite M2K Pitampura, Delhi – 110034 (INDIA).
Ph: +91-11-42208000, 42208111, 42208222
Mobile: +91-9810521400
Fax: +91-11-42208444
Email: customerservice@atzlabs.com
Web: www.atzlabs.com

INTENDED USE

The Bovine Albumin ELISA Kit is a competitive immunoassay for the quantification of bovine serum albumin (BSA) in serum, or in solutions with high BSA content.

INTRODUCTION

Albumin is synthesized by the liver using dietary protein, and is the protein of the highest concentration in plasma. 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. Albumin also transports many small molecules in the blood, including fatty acids, bilirubin, calcium, progesterone, and many drugs. Liver disease, kidney disease, and malnutrition are the major causes of low albumin. A diseased liver produces insufficient albumin. Diseased kidneys sometimes lose large amounts of albumin into the urine faster than the liver can produce it (this is termed nephrotic syndrome).

Plasma albumin concentration is an important indicator of nutritional status, and low concentrations pre-surgery increase the risk of post-operative wound dehiscence, seroma formation and infection. Albumin levels are also dependant on the state of hydration of the body. An individual that is dehydrated will have an artificially high albumin level, which would return to normal when the dehydration is corrected. Albumin fluctuates so widely because it is very sensitive to changes in hydration of the body

PRINCIPLE OF THE TEST

The Bovine Albumin ELISA kit is based on competitive binding of fixed concentrations of anti-BSA-HRP conjugate to BSA coated on the plate and to BSA in the samples. Higher concentrations of BSA in the samples reduce the amount of anti-BSA-HRP that binds to the plate. After a washing step, chromogenic substrate is added and color is developed. The enzymatic reaction (color) is indirectly proportional to the amount of albumin present in the sample, the higher the concentration of BSA in the sample the lower the signal. Adding stop solution terminates the reaction. Absorbance is then measured on a microtiter well ELISA reader at 450 nm and the concentration of albumin in samples and control is read off the standard curve.

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.

Sensitivity

Based on 5 replicate determinations of the zero standards, the minimum albumin concentration detectable using this assay is 250 ng/ml. The detection limit is defined as the value deviating by 2 SD from the zero standard.

Normal Range

A limited testing of pooled bovine serum samples gave values of 11-22 mg/ml (average 15.5mg/ml).

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

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 label. Stabilities of the working solutions are indicated under Reagent Preparation.

CALCULATION OF RESULTS

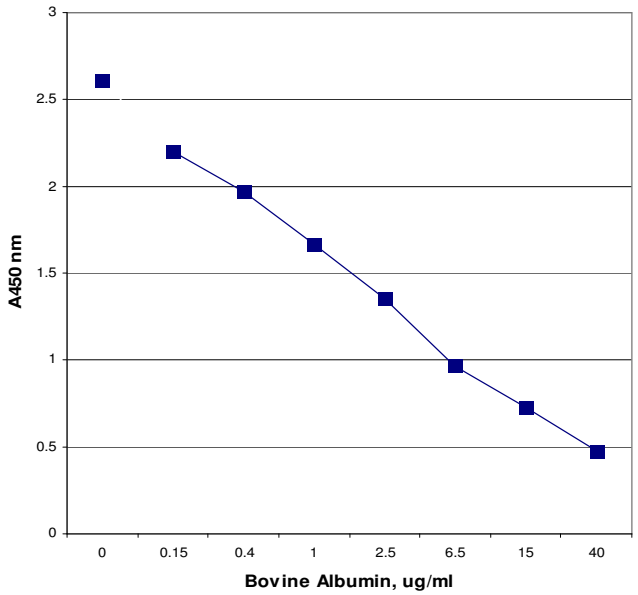
The results may be calculated using any immunoassay software package. The four-parameter curve-fit is recommended. If software is not available, Bovine Albumin concentrations may be determined as follows:

1. Calculate the mean OD of duplicate samples.
2. On graph paper plot the mean OD of the standards (y-axis) against the concentration (ug/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 40 ug/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 ug/ml
1A, B	**Negative Diluent Control**	2.61	0
1C, D	0.15 ug/ml **Standard**	2.20	0.15
1E, F	0.4 ug/ml **Standard**	1.97	0.4
1G, H	1.0 ug/ml **Standard**	1.67	1.0
2A, B	2.5 ug/ml **Standard**	1.35	2.5
2C, D	6.5 ug/ml **Standard**	0.97	6.5
2E, F	15 ug/ml **Standard**	0.73	15
2G, H	40 ug/ml **Standard**	0.48	40



KIT CONTENTS

Ready For Use: Store as indicated on labels.

Component	Part #	Amt	Contents
Bovine Albumin Microwell Strip Plate	8001	8-well strips (12)	Coated with purified Bovine Albumin. Return unused strips to the pouch with desiccant; re-seal and store refrigerated.
Bovine Albumin Standards			
0.15 ug/ml 0.4 ug/ml 1.0 ug/ml 2.5 ug/ml 6.5 ug/ml 15 ug/ml 40 ug/ml	8003A 8003B 8003C 8003D 8003E 8003F 8003G	0.25 ml 0.25 ml 0.25 ml 0.25 ml 0.25 ml 0.25 ml 0.25 ml	Seven (7) vials, each containing the specified concentration of BSA; diluted in buffer with detergents and ProClin 300 as stabilizers.
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 2-8°C 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. 8004, 0.15ml	Peroxidase conjugated anti-bovine serum albumin (BSA) antibody in buffer with protein, detergents and ProClin 300 as stabilizers. Dilute fresh as needed; 10ul of concentrate to 1ml of **Working Sample 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 2-8°C storage.

Materials Required But Not Provided:

- Pipettors and pipettes that deliver 100ul and 1-10ml. A multi-channel pipetter 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. Distilled or deionized water to dilute reagent concentrates.

PRECAUTIONS AND SAFETY INSTRUCTIONS

Standards, Sample Diluent, and HRP Antibody contain Proclin 300 (0.05%, v/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 Proclin 300, if not already on file, can be requested or obtained from ADI or its website.

SPECIMEN COLLECTION AND HANDLING

Culture medium, serum and other biological fluids may be used as samples with proper dilution to avoid solution matrix interference. For serum, collect blood by venipuncture, allow clotting, and separate the serum by centrifugation at room temperature. For other samples, including tissue culture media, clarify the sample by centrifugation and/or filtration prior to dilution in Working Sample Diluent. If samples will not be assayed immediately, stored refrigerated for up to a few weeks, or frozen for long-term storage. Avoid freeze-thaw cycles.

QUALITY CONTROL

Reagents Accurate and reproducible assay results rely on proper storage, handling and control of reagent and sample temperature. Store all reagents as indicated, and warm to room temperature only those to be used in the assay. Shelf-life of the critical reagents and samples will diminish with extended exposure to non-refrigeration, resulting in inaccurate assay results. All solutions should be clear. Cloudiness or particulates are indications of reagent contamination or instability and may interfere with proper performance of the assay. Do not use.

Standard Curve In this competitive assay, signal generated by the Standards should be continuously decreasing in OD from the lowest Standard to the highest Standard, with a difference greater than 1.2 OD. Non-continuously decreasing or low signals may indicate problems with technique, protocol directions and/or reagent preparation, use or stability. A Negative Diluent Control should be of higher signal than the lowest standard. Do not rely on results generated from an assay with these issues.

ASSAY PROCEDURE

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

DILUTE Serum Samples in Working Sample Diluent. Dilutions of at least 1000-fold are appropriate for most normal bovine sera. For accuracy, two dilution steps are recommended, as follows:

1) 20ul serum + 780ul diluent = [1:40],
2) 20ul [1:40] + 480ul diluent = **[1:1000].**

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. Sample Addition [20ul

- Add 20ul of standards, samples and controls each to pre-determined wells.

Conjugate Addition + 80ul – 60min]

- Immediately: add 80ul of Working Anti-BSA-HRP Conjugate to each well.
- Tap the plate gently to mix reagents and incubate for 60 minutes.
- Wash wells 5 times and pat dry on fresh paper towels.

3. Substrate Incubation [100ul - 15min]

- Add 100ul TMB Substrate to each well. The liquid in the 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, or read OD at 405-410 nm (results are valid).

4. 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.

5. 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.