Bovine Serum Albumin

ELISA Kit

Enzyme Immunoassay for the quantification of Bovine Serum Albumin (BSA) in biological fluids

Catalog number: ARG81124

Package: 96 wells

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MANUFACTURED BY:

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INTRODUCTION

Albumin is a soluble, monomeric protein which comprises about one-half of the blood serum protein. Albumin functions primarily as a carrier protein for steroids, fatty acids, and thyroid hormones and plays a role in stabilizing extracellular fluid volume. Albumin is a globular unglycosylated serum protein of molecular weight 65,000. Albumin is synthesized in the liver as preproalbumin which has an N-terminal peptide that is removed before the nascent protein is released from the rough endoplasmic reticulum. The product, proalbumin, is in turn cleaved in the Golgi vesicles to produce the secreted albumin. [provided by RefSeq, Jul 2008]

PRINCIPLE OF THE ASSAY

This is a two-step Enzyme Immunoassay for the quantification of Bovine Serum Albumin (BSA) in biological fluids.

A highly specific anti-BSA antibody has been pre-coated onto a microtiter plate. Standards or samples are pipetted into the wells. After incubation, the unbound components are washed away. Next, Horseradish Peroxidase (HRP) labeled anti-BSA antibody are added and sandwich complex are formed together with the immobilized components. Unbound components are removed by washing with 1X washing buffer. Then substrate solution (TMB) reactive with HRP is added to the wells. A colored product is formed in proportion to the amount of BSA present in the sample or standard. The reaction is terminated by addition of acid and absorbance is measured at 450 nm. The absorbance of the unknown samples can be transformed into their corresponding BSA concentrations by reading from the standard curve.

MATERIALS PROVIDED & STORAGE INFORMATION

Store the unopened kit at 2-8 °C. Use the kit before expiration date.

Component	Quantity	Storage information
Antibody-coated microplate	12 strips x 8 wells	4°C
10X Wash buffer	50 ml	4°C
Sample Diluent	70 ml (Ready-to-use)	4°C
Standard 1-6	1 ml X 6 vials (Ready-to-use) S1: 500 ng/ml S2: 250 ng/ml S3: 125 ng/ml S4: 60 ng/ml S5: 30 ng/ml S6: 15 ng/ml	4°C
Control	1 ml (Ready-to-use) (200 ng/ml; accept range: 150-250 ng/ml)	4°C
101X HRP-conjugate	0.3 ml	4°C
TMB substrate	15 ml (Ready-to-use)	4°C (Protect from light)
STOP solution	15 ml (Ready-to-use)	4°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 450 nm and a ≥ 620 nm as the reference wavelength.
- Pipettes and pipette tips
- Deionized or distilled water
- Automated microplate washer (optional)

TECHNICAL HINTS AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Store the kit at 4°C at all times.
- Once opened all kit components are stable for up to 2 months under appropriate storage conditions at 2-8°C.
- Allow the sealed plate to reach room temperature before opening. Unused wells should be stored refrigerated and protected from moisture in the original cover carefully resealed.
- Briefly spin down the reagents before use.
- If crystals are observed in the 10X Wash buffer warm to RT or 37°C (not more than 50°C) until the crystals are completely dissolved.
- The Sample Diluent is ready-to-use. During storage at 2-8°C precipitates may occur, that will dissolve when the solution is warmed to room temperature. Make sure the Sample Diluent has reached room temperature before starting the assay.
- Ensure complete reconstitution and dilution of reagents prior to use.
- It is highly recommended that the standards, samples and controls be assayed in duplicates.
- All materials should be equilibrated to room temperature (RT) before use.
- Change pipette tips between the addition of different reagent or samples.

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Cell Culture Supernatants - Remove particulates by centrifugation at 4°C and aliquot & store samples at ≤ -20°C. Avoid repeated freeze-thaw cycles.

Serum- Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation at 4°C for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Plasma - Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge at 4°C for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Note: Frozen samples should be rapidly warmed to room temperature and mixed thoroughly before testing.

REAGENT PREPARATION

- 1X Wash buffer: Dilute 10X Wash buffer into distilled water to yield 1X Wash buffer. The diluted wash buffer is stable for at least 30 days when stored at 2-8°C.
- Samples: Dilute samples with Sample Diluent (usually 1:6 to 1:21 dilution. For 1:6 dilution: add 200 µl sample into 1000 µl Sample Diluent. For 1:21 dilution: add 50 µl sample into 1000 µl Sample Diluent). The dilution varies in dependence from the materials and expected BSA concentration. For the calculation of the concentrations this dilution

factor has to be taken into account. (It is recommended to do pre-test to determine the suitable dilution factor).

- **HRP Conjugate:** Dilute 101X HRP conjugate concentrate with Sample Diluent to yield 1X working solution. (e.g., 10 µl of HRP conjugate concentrate + 1000 µl of Sample Diluent). Prepare 1 ml working solution for each test strip. Prepare the 1X HRP conjugate solution at least 15 min prior to use. The 1X HRP conjugate solution is stable for 1 day at room temperature.

ASSAY PROCEDURE

Attention: This BSA ELISA kit is a very sensitive assay detecting less than 15 ng BSA per ml of sample. It is recommended to use new disposable reagent containers for pipetting the reagents. Make sure that the glassware used for buffer preparation and the equipment used for running the assay are absolutely free of BSA. Any possibility of contamination of working equipment with BSA should be strictly avoided! Therefore special dispensers, pipettes and washers have to be used only for this test and not for other ELISAs.

All materials should be equilibrated to room temperature (RT, 20-25°C) before use. Standards, samples and controls should be assayed in duplicates.

1. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal it.
2. Add **100 µl** of **diluted samples, control or standards** to the respective wells.
3. Cover wells and incubate for **1 hours at RT**.
4. Aspirate each well and wash, repeating the process 4 times for a total **5**

washes. Wash by filling each well with **1× Wash Buffer (300 µl)** using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating, decanting or blotting against clean paper towels.

5. Add **100 µl** of **1X HRP-conjugate** working solution into each wells.
6. Cover wells and incubate for **1 hours at RT.**
7. **Wash** as according to step 4.
8. Add **100 µl** of **TMB substrate** to each well. Incubate for **15 minutes at room temperature in dark**.
9. Add **100 µl** of **Stop Solution** to each well.
10. Read the OD with a microplate reader at **450 nm** (and ≥ 620nm as the reference wavelength) immediately, it is recommended to read the O.D. within 30 min after adding Stop solution.

CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of standards, controls and samples.

2. Using semi-log or linear graph paper, construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.

3. Using the mean absorbance value for each sample determine the corresponding concentration from the standard curve.

4. Automated method: The results in the IFU have been calculated automatically using a 4 PL (4 Parameter Logistics) curve fit. 4 Parameter Logistics is the preferred method. Other data reduction functions may give slightly different results.

5. arigo provides GainData®, an in-house development ELISA data calculator, for ELISA data result analysis. Please refer our GainData® website for details. (https://www.arigobio.com/elisa-analysis)

6. If the samples have been diluted, the concentration read from the standard curve must be further converted by the appropriate dilution factor according to the sample preparation procedure as described above.

7. Test Validity: A test run is valid if:

(a) The mean absorbance of standard 1 (500.0 ng/ml) is ≥ 1.50

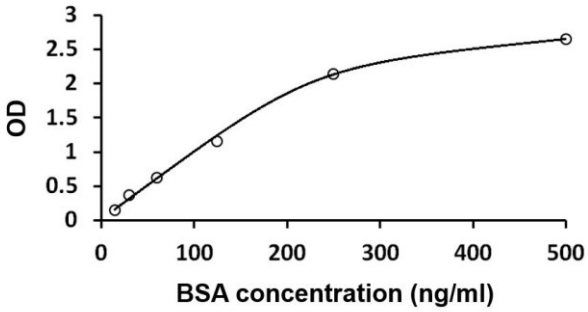
(b) The mean absorbance of standard 6 (15.0 ng/ml) is ≤ 0.50

(c) The mean of control is determined between 150-250 ng/ml

If the above mentioned quality criteria are not met, repeat the test and make sure that the test procedure is correct (incubation times and temperatures, sample and wash buffer dilution, washing steps etc.).

EXAMPLE OF TYPICAL STANDARD CURVE

The following data is for demonstration only and cannot be used in place of data generations at the time of assay.



QUALITY ASSURANCE

Sensitivity

The minimum detectable dose (MDD) of BSA was 15 ng/ml.

The assay range was 15 – 500 ng/ml.

Intra-assay and Inter-assay precision

The CV value of intra-assay precision was 2.33-9.94% and inter-assay precision was 5.89-10.03%.

Specificity

ARG81124 Bovine Serum Albumin ELISA Kit reacts to Bovine Serum Albumin and it has been tested to cross-reacts to sheep serum albumin. And there is no significant cross-reactivity for 5 ng/ml –1000 ng/ml of Human serum albumin, Ovalbumin and rabbit albumin.