ab285245 – BSA ELISA Kit

For the quantitative measurement of BSA in human serum, plasma, culture supernatants and other biological fluids.
For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit:
http://www.abcam.com/ab285245

Storage and Stability

The entire ELISA kit may be stored at 4°C for up to 6 months from the date of shipment.

Materials Supplied

Item	Quantity	Storage Condition
Micro ELISA Plate	8 x 12 Strips	4°C
Standard (400 ng/mL)	0.6 mL	4°C
Standard Diluent	6 mL	4°C
Special Diluent	6 mL	4°C
HRP-Conjugate Reagent	6 mL	4°C
Chromogen Solution A	6 mL	4°C
Chromogen Solution B	6 mL	4°C
Stop Solution	6 mL	4°C
Wash buffer (20X)	20 mL	4°C
Plate sealers	2 units	4°C

Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully utilize this assay:

- Microplate reader capable of measuring absorbance at 450 nm
- 37°C incubator
- Clean eppendorf tubes for preparing standards or sample dilutions
- Absorbent paper
- Deionized or dilstilled water

Reagent Preparation

- Prepare reagents within 30 minutes before the experiment.
- Before using the kit, spin tubes and bring down all components to the bottom of tubes.

Wash Buffer: Dilute Concentrated Wash Buffer (20X) into 19 parts of deionized or distilled water. Put unused solution back at 4°C. If crystals have formed in the concentrate, warm it with 40°C water bath and mix it gently until the crystals have completely dissolved. The solution should be cooled to room temperature before use.

Standard Preparation

1. Prepare 0.6 ml of 200 ng/ml standard by adding 0.3 ml of the 400 ng/ml stock solution in 0.3 ml of Standard Diluent. Perform 2-fold serial dilutions of the top standards to make the standard curve within the range of this assay. Use 0.3 ml standard diluent as blank control.
2. Suggested standard points are 400, 200, 100, 50, 25, 0 ng/ml

Sample Preparation

- Samples to be used within 5 days may be stored at 4°C, otherwise samples must be stored at -20°C (≤1 month) or -80°C (≤2 months) to avoid loss of bioactivity and contamination.
- Avoid multiple freeze-thaw cycles.
- Samples that contain NaN_3 cannot be used for this assay.
- Do not use heat-treated samples.

Serum: Collect blood with non-pyrogenic and endotoxin tubes to avoid any cell stimulation. Centrifuge 3000 rpm for 10 minutes and separate the serum and red blood cells as quickly as possible. If precipitation appears, centrifuge again.

Plasma: Collect plasma with EDTA, heparin sodium or sodium citrate as the anticoagulant. Mix for 20 minutes and centrifuge for 30 min at 2-8°C at 3000 rpm. For eliminating the platelet effect, suggesting that further centrifugation for 10 min at 2-8°C at 10000xg. Collect the supernatant and carry out the assay immediately. Avoid hemolysis, high cholesterol samples.

Tissue Samples: After cutting samples, check the weight, add PBS (pH7.4), Rapidly freeze with liquid nitrogen, keep samples at 2-8°C after melting, add PBS, Homogenize by hand or grinders, centrifuge for 20 minutes at 2000-3000 rpm. Collect supernatant.

Cell culture supernatant: Centrifuge supernatant for 20 minutes at 2000 – 3000 rpm to remove insoluble impurity and cell debris. Collect the clear supernatant and carry out the assay immediately or aliquot and store at -20°C.

Δ Note: *End user should estimate the concentration of the target protein in the test sample first and select a proper dilution factor to make the diluted target protein concentration fall in the optimal detection range of the kit.*

Assay Procedure

- Bring all reagents and samples to room temperature 30 minutes prior to the assay.
- It is recommended that all standards and samples be run at least in duplicate.
- A standard curve should be run for each assay.

1. Prepare all reagents, samples, and standards.
2. Set blank wells, standard wells, and test sample wells respectively:
    a. Blank well: do not add samples and HRP-Conjugate reagent, other operations are the same.
    b. Standard wells: Add standard 50 µl to Standard wells.
    c. Test sample wells: Add 40 µl of Special diluent and then add 10 µl of samples. (The final sample dilution is five times and the final result calculation should be multiplied by five times).
    d. Add 50 µl of HRP-Conjugate reagent into each well, except blank well. Then seal the plate, and gently shake, then incubate 60 minutes at 37°C.
3. Discard the solution and wash 5 times with 1X Wash Solution. Wash by filling each well with Wash Buffer (350 µl) using a multi-channel pipette or autowasher. Let it soak for 1-2

minutes, and then remove all residual wash-liquid from the wells by aspiration. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Clap the plate on absorbent filter papers or other absorbent materials.

4. Add 50 µl of Chromogen solution A to each well, and then add 50 µl of Chromogen solution B to each well. Gently shake and incubate for 10 minutes at 37°C away from light.
5. Add 50 µl Stop Solution into each well to stop the reaction.
6. Measure the optical density (OD) at 450 nm wavelength within 15 minutes after adding the stop solution.

Calculations

Set blank well zero, measure the optical density (OD) at 450 nm. The standard curve can be plotted as the relative O.D.450 of each standard solution (Y) vs. the respective concentration of the standard solution (X). The BSA concentration of the samples can be interpolated from the standard curve. If the samples measured were diluted, multiply the dilution factor to the concentrations from interpolation to obtain the concentration before dilution.

Download our ELISA guide for technical hints, results, calculation, and troubleshooting tips:
www.abcam.com/protocols/the-complete-elisa-guide

For all technical or commercial enquiries please go to:

www.abcam.com/contactus
www.abcam.cn/contactus (China)
www.abcam.co.jp/contactus (Japan)