Bovine Serum Albumin (BSA) ELISA Kit

48 Well

Catalogue Number: NEGES0014-48T

Valid Period: 6 months

For samples:

ELISA Kit for the quantitative Measurement of Bovine Serum Albumin Residues in Protein Purification Process, and End-Product (purified fermentation broth, cell culture supernatant, etc.)

FOR RESEARCH USE ONLY!

NOT FOR THERAPEUTIC OR DIAGNOSTIC APPLICATIONS!

PLEASE READ THROUGH ENTIRE PROCEDURE BEFORE BEGINNING!

CONTENTS

1. BACKGROUND

Bovine Serum Albumin (BSA) is a primary protein component found in bovine serum, with a molecular weight of approximately 66kDa. It is a single-chain protein with multiple secondary and tertiary structures, highly soluble in water, and capable of binding small molecules like fatty acids, hormones, and drugs.

In protein experiments, BSA is commonly used as a stabilizer to prevent aggregation and denaturation of target proteins. It is also employed as a standard in ELISA and other bioassays and can serve as a component in antigen or antibody dilution buffers. In cell culture, BSA is widely added to media to provide essential amino acids and nutrients, making it commonly used in biochemical research.

However, BSA can bind to target recombinant proteins, interfering with their proper conformation and biological function. In various immunoassays (such as ELISA and Western Blot), BSA can contribute to nonspecific background interference, complicating data analysis. Residual BSA in cell culture experiments can affect cell proliferation, differentiation, and other biological responses, especially in drug screening and bioassays.

BSA can also cause immune reactions, leading to allergic responses in humans, which can impact drug clinical applications and product safety. The allergic mechanism involves IgE-mediated and non-IgE-mediated pathways. When BSA is initially recognized as a foreign protein by the immune system, B cells become activated and produce specific IgE antibodies. These IgE antibodies bind to high-affinity IgE receptors on mast cells and basophils. Upon subsequent exposures, BSA binds to these cell-surface IgE antibodies, causing degranulation of mast cells and the release of bioactive substances like histamine, leukotrienes, and cytokines. These mediators induce vasodilation (causing local redness and inflammation), smooth muscle contraction (causing respiratory symptoms like asthma, rhinitis, or difficulty breathing), and nerve stimulation (causing itching and skin reactions such as urticaria or eczema), or digestive symptoms (such as nausea and vomiting). In severe cases, it may lead to anaphylactic shock.

In summary, BSA-induced allergic reactions involve a complex immune process in response to this exogenous protein. Despite purification steps, residual BSA may still be present in recombinant protein drugs and final products. Therefore, biopharmaceutical manufacturers must test for BSA

content in both in-process materials and finished products.

According to the 2015 edition of the Pharmacopoeia of the People's Republic of China, BSA content in biopharmaceutical products should not exceed 50ng/dose. This kit utilizes a double-antibody sandwich ELISA, with specific antibodies coated to ensure selective binding. The kit's advantages include short operation time, high sensitivity (as low as 0.03ng/mL), and a low detection range (0.5–32ng/mL), facilitating monitoring of residual BSA in biopharmaceuticals to enhance quality control in production and ensure final product safety.

2. PRINCIPLE OF THE ASSAY

This ELISA kit is applied to the double antibody sandwich Enzyme Linked Immunosorbent Assay to detect the concentration of BSA in samples. The microtiter plate has been pre-coated with anti-BSA antibody , standards or samples are then added to the microtiter plate wells and BSA if present, will bind to the antibody pre-coated wells under specific conditions. Then wash the plate to remove unbound substances, add the HRP-conjugated antibody, and incubate at room temperature (20-25°C) to form a double-antibody sandwich complex with the coated antibody, BSA, and detection antibody. Then a TMB substrate solution is added to the wells to incubate. TMB substrate solution turns blue after the oxidation of HRP, and finally turns to yellow immediately after adding the stop solution. Color of TMB substrate positively correlated with total BSA bound in the initial steps. The color is measured by spectrophotometrically with wavelength of 450nm. The concentration of BSA in samples is then determined by comparing the O.D. of the samples to the standard curve.

3. KITS' ADVANCEMENT

1) High Specificity: Capture antibody and detection antibody respectively identify different epitopes of the antigen, maximizing the specificity of the reaction, with no cross-reactivity to HSA, Fish Gelatin.
2) High Stability: The experiment uses high-quality coated antibodies and antigens, and also applies the broad-spectrum protein stabilizers, and antibody pre-coated microplate treatment to increase the thermostability of the microplate, and reproducibility of results.

4. MATERIALS AND EQUIPMENTS (Note: Storage at 2-8℃)

	REAGENTS	SPECIFICATION	QUANTITY
1	Pre-Coated Microplate (Detachable)	48 wells	1 plate (Keep Sealed)
2	Standard (Stock Solution) -10μg/mL	10μL	1 vial
3	HRP-conjugated antibody (200×)	50μL	1 vial
4	TMB Substrates	10mL	1 vial (Avoid Light)
5	Stop Solution	10mL	1 vial
6	Wash Solution (100×)	10mL	1 vial
7	Diluent Buffer (10×)	10mL	1 vial
8	Plate Sealer		4 pieces
9	Instruction Manual		1

5. EQUIPMENT REQUIRED BUT NOT PROVIDED

1) 10-1000μL pipettor and disposable sterilized tips
2) Multichannel pipettor
3) 1L sterilized deionized water or ultrapure water
4) Sterilized EP tubes
5) Absorbent Paper
6) Microplate reader
7) High-speed centrifuge
8) Microplate washer or washing bottles
9) Incubator or water bath (37°C/80°C)
10) Data analysis and graphing software
11) Preparation of PBS: NaH_2PO_4 0.2g, $Na_2HPO_4 \cdot 12H_2O$ 2.9g, NaCl 8g，KCl 0.2g. Then dilute these to 1000mL with distilled water.

6. SPECIMEN COLLECTION AND STORAGE

Cell Culture Supernatant -Collect cultured cell, centrifuge them at 1000×g (or 3000 rpm) for 15 minutes, and collect the supernatant for immediate assay or store samples in aliquots at -20°C or -80°C.

NOTE:

- Samples should be aliquoted and must be stored at -20°C (less than 3 months) or -80°C (less than 6 months) to avoid loss of bioactivity and contamination. If samples are to be run within 24 hours, they may be stored at 2- 8°C. Avoid repeated freeze-thaw cycles.
- Some chemical solvents may interfere with this experiment, such as SDS, Triton, etc.
- The precipitates in the sample solution can interfere with ELISA. Make sure to centrifuge and remove them.
- Do not use heat-treated specimens.

7. PREPARATION BEFORE ASSAY

1) Please read through this manual carefully before using, and set the temperature at 37°C for Incubator or Water bath.
2) The frozen samples should be slowly restored to room temperature and then mix well, centrifuge the samples at 12000 rpm for 1 minute. Collect the supernatant for immediate assay.
3) Sample Dilution: Please predict the concentration before assaying. If the predicted concentration of your sample exceeds the maximum detection limit of this kit, it is recommended to dilute the sample with PBS.

NOTE:

- If the samples are not indicated in the manual, a preliminary experiment to determine the validity of the kit is necessary.
- Fresh samples without long time storage are recommended for the test. Otherwise, protein degradation and denaturalization may occur in those samples, and lead to inaccurate results.
- The optimal pH value of the sample is between 7.0-7.4.

8. REAGENT PREPARATION

1) Place all kits' components at room temperature for 30mins before using.
2) The preparation of Wash Solution (1×): Dilute 10mL of Wash Solution (100×) with 990mL of deionized water or ultrapure water to prepare 1000mL of Wash Solution (1×). If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have

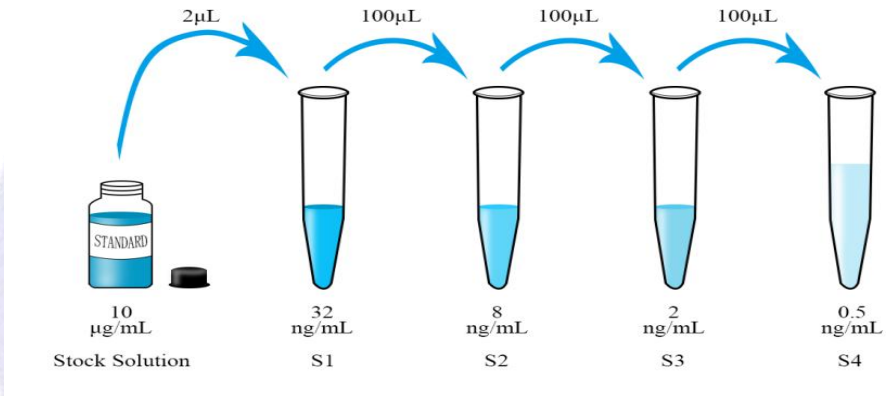
completely dissolved. Wash Solution (1×) can be stored at 2-8°C for 2 weeks.

3) The preparation of Diluent Buffer: Dilute 10mL of Diluent Buffer (10×) with 90mL of deionized water or ultrapure water to prepare 100mL of Diluent Buffer (1×) . If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved.

4) Prepare Standards: When preparing the standards, label 4 tubes, S1-S4 and add a certain volume of diluent: S1(623µL), S2 to S4 (each 300µL).

Add 2µL of the Standard (Stock Solution-10µg/mL) into the tube labeled as S1. Shake well and then pipette 100µL S1 (32ng/mL) from S1 to S2. And then produce a 4-fold dilution series until S4.

NOTE: There are 4 points of diluted standards, S1: 32ng/mL, S2: 8ng/mL, S3: 2ng/mL, S4: 0.5ng/mL.



5) Preparation of HRP-conjugated antibody (1×): Extract 25µL of HRP-conjugated antibody (200×), and add it into 5mL Diluent Buffer (1×), and mix well.

9. ASSAY PROCEDURES

1) Remove the microplate from the foil bag, detach the desired number of strips from the plate, immediately reseal the remaining strips and store at 4°C. (Note: The microplate frame can be reused).

2) Secure the desired plate in the holder then add 100µL of diluted standards (S1-S4) or 100µL (diluted or undiluted) samples to appropriate wells. For the Blank Control test, we recommend add 100µL Diluent Buffer to the well.

3) Cover the plate with sealer, put it on the oscillator to mix and incubate for 90 mins at room

temperature.

4) Automated Washing:

- Put 1000mL Wash Solution (1×) into the washing bottle of the automated microplate washer for standby.
- Take out the microplate, and discard the incubation mixture. And then wash the microplate with the automated microplate washer for 5 times.
- After washing, invert plate and blot dry by hitting the plate onto absorbent paper or paper towels until there is no moisture appears.

Or Manual Washing:

- Put 1000mL Wash Solution (1×) into the washing bottle for standby.
- Take out the microplate, and discard the incubation mixture. And then invert plate and blot dry by hitting the plate onto absorbent paper or paper towels until there is no no moisture appears.
- Fill each well with 300μL Wash Solution (1×) by a muti-channel pipette, let stand for 20 seconds, then discard the contents, invert plate, and blot dry by hitting the plate onto absorbent paper. Repeat this washing step for 5 times. Note: There should be no moisture appears in the fifth washing step.

5) Add 100μL of HRP-conjugated antibody (1×) to each wells, cover the plate with a sealer, and then incubate for 90 mins at room temperature. Repeat the wash process as above Step 4.

6) Add 100μL TMB substrate to each well, cover the plate with a plate sealer, incubate at room temperature for no more than 10mins. (Avoid sunlight).

Note: The coloring time varies in different experimental conditions (temperature, humidity, ect.).

7) Add 50μL of Stop Solution to each well to stop the reaction, mix well. Immediately run the microplate reader and conduct measurement at 450nm.

10. NOTES

1) **Sample Preparation**

a) After collection, the samples should be aliquoted and stored at -20°C (less than 3 months) or -80°C (less than 6 months) to maintain protein activity, avoid contamination and multiple

freeze-thaw cycles. If samples are to be run within 24 hours, they may be stored at 2- 8°C.

b) Samples should be frozen and aliquoted if not analyzed shortly after collection. Avoid multiple freeze-thaw cycles of frozen samples. Thaw completely and mix well by pipette or Vortex, centrifuge to remove flocculent insoluble substances before use.

c) If turbidity appears in the stock solution (Standard, Detection antibody and HRP-Streptavidin), mix gently or pipette up and down for several times.

d) It is recommended that all standards, controls and samples be run in duplicate. Please gently mix the liquid after adding standards and samples, and confirm there's no bubble inside.

e) Do not use hyperlipidemia or hemolysis samples, which may interfere with ELISA and lead to inaccurate results.

2) Experimental Operation

a) Do not mix or interchange different ELISA kits or same ELISA kit but with different lot.

b) To avoid cross-contamination, change pipette tips between different diluted standard additions, sample additions, and reagent additions.

c) Total dispensing time for addition of reagents or samples to the plate should not exceed 10 minutes. When pipetting reagents, maintain a consistent order of addition from well-to-well. This ensures equal incubation times for all wells.

d) Incomplete washing will adversely affect the test outcome. All washing must be performed with Wash Solution provided. All residual wash liquid must be drained from the wells by efficient aspiration or by decantation followed by tapping the plate forcefully on absorbent paper. Never insert absorbent paper directly into the wells.Do not directly touch the bottom of the microplate. If it is covered with fingerprints or stains, it will affect the measurement.

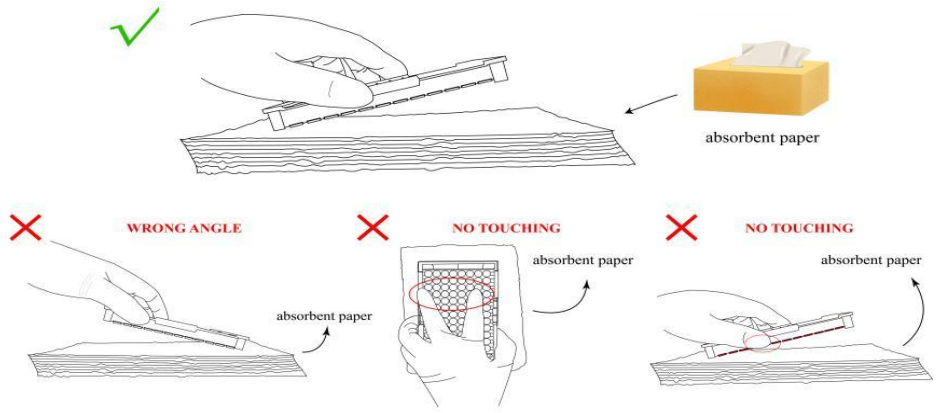
e) TMB is easily contaminated. please protect it from light and metal.

f) Monitor the reaction time. After adding TMB substrate, observe the color change of reaction wells at regular intervals (for example, about 10 minutes). If the color turns too deep, add Stop Solution in advance to terminate the reaction.

g) The Stop Solution provided with this kit is acid solution. Protective gloves, clothes, and face protection should be worn.

h) The coefficient of determination of the standard curve should be $R^2 \geqslant 0.95$.

i) Schematic diagram of tapping the plate:



11. CALCULATION OF RESULTS

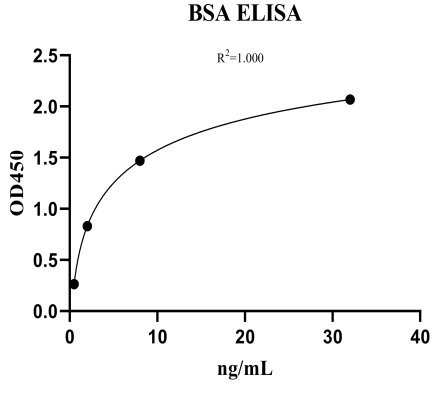
1) Average the duplicate readings for each standard and sample. All O.D. values are subtracted by the mean value of blank control before result interpretation.

2) Construct a standard curve by plotting the average O.D. for each standard on the vertical (Y) axis against the concentration on the horizontal (X) axis, and generate a four paramater logistic (4-PL) curve-fit.

3) Calculate the concentration of samples corresponding to the mean absorbance from the standard curve.

4) Standard curve for demonstration only.

APPENDIX 1 : EXAMPLE OF STANDARD CURVE



ng/mL	OD450
32	2.068
8	1.470
2	0.830
0.5	0.263

12. QUALITY CONTROL

1) Intra Variation%: 4-8

2) Inter Variation%: 8-10

3) Sensitivity:

LOD: 0.03ng/mL

LOQ: 0.5ng/mL

4) Specificity/Cross-reactivity:

Sample Type	Cross Reactivity（%）
HSA	NA
Fish Gelatin	NA
Gelatin (Porcine/Bovine)	6%

5) Hook Capacity: This kit is a two-step sandwich ELISA kit. Samples with high BSA concentrations may be affected by the hook effect. Please dilute the sample using the kit's diluent to bring the BSA concentration within the kit's detection range.

8) Limitation: This ELISA kit is not suitable for the samples containing NaN_3. NaN_3 is a strong inhibitor for HRP that will decrease the concentration of the target protein.

13. SAFETY NOTES

1) This kit contains small amount of 3, 3', 5, 5'- Tetramethylbenzidine (TMB) in Substrate B. TMB is non-carcinogenic but it is hazardous in case of skin contact, eye contact, ingestion and inhalation. In case of contact, rinse affected area with plenty of water.

2) The Stop Solution provided with this kit is an acid solution. Wear protective gloves, clothing, and face protection.

14. CONTACT US

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