

BSA ELISA Kit

Product description

The production of biologics often involves cell culture or tissue culture, in which fetal bovine serum is widely used to provide essential nutrients for cell growth, and fetal bovine serum contains a certain amount of BSA (Bovine serum albumin). Considering the safety of the drugs produced, serum-free media are gradually used to replace serum-containing media in the production of biologics such as vaccines, antibody drugs, tissue-engineered products, and cells & GCTs, so as to minimize potential hazards such as viral contamination that may be brought about by serum. Among them, serum-free culture media are mostly formulated with various biomolecules that can replace the function of serum, such as bovine serum albumin (BSA), transferrin and insulin. As a result, BSA is present in both serum-containing and serum-free media, and although most of the impurity proteins are removed by purification and other steps in production, trace amounts of exogenous proteins (e.g., BSA, etc.) are still present in the final bioproducts.

BSA, as an exogenous protein, may cause serious allergic reactions after entering the human body, thus affecting the safety of biologics. The 2020 edition of the Chinese Pharmacopoeia (Part III) specifies the residual amount of BSA in various vaccines, which is basically no more than 50 ng/mL or no more than 50 ng/dose.

ELISA is a simple to use, sensitive and objective method for residue detection of BSA. This kit uses the principle of double-antibody sandwich enzyme-linked immunosorbent assay (sandwich ELISA) to detect the residual BSA in samples. First, add the BSA standard (36722-B) and test sample to the Anti-BSA coated microtiter strips (36722-A), and then diluted HRP-labeled BSA detecting antibody (36722-C) is added to form an antibody + antigen + antibody-HRP complex. HRP complex, wash the plate and add TMB color development mix (36722-E + 36722-F) to develop the color. TMB is catalyzed by HRP enzyme to change from colorless to blue and finally to yellow under the action of the termination solution (36722-G). The shade of yellow color is positively correlated with the amount of BSA detected in the sample. This kit can be used for the optimization of biologics purification processes, impurity control in intermediate processes, and final product release testing.

The detection range of this kit is 1.5625 ng/mL~50 ng/mL; the lower detection limit is ≤ 1 ng/mL.

Specifications

Cat.No.	36722ES96
Size	96 T

Components

Components No.	Name	36722ES96
36722-A	Anti-BSA coated microtiter strips	96 T
36722-B	Standard: BSA (5 μ g/mL)	300 μ L
36722-C	Detection Antibody: HRP-conjugated Antibodies (1250 $\times$)	40 μ L
36722-D	Dilution&Wash Buffer (20 $\times$)	30 mL
36722-E	TMB Substrate A	8 mL

36722-F	TMB Substrate B	8 mL
36722-G	Stop Solution	15 mL
36722-H	Plate Sealer	3 each

Storage

This product should be stored at 2°C ~8°C. Unopened product is valid for one year.

*Upon receipt of the kit, please check whether all components are complete and immediately store them in corresponding condition.

Instructions

1. Preparations before the experiment

1) Materials required but not provided:

- Graduated cylinder, Beaker, Centrifuge tube of different volumes;
- Dust-free paper (used for plate washing)
- Sterile pipette tips;
- Pre-treated plates;
- Deionized water or double distilled water.

2) Instruments and equipment required but not provided:

- 37°C incubator;
- Plate washer;
- Timer, Vortex and Centrifuge;
- Single channel or multi-channel pipettes;
- 2°C ~8°C refrigerator;
- Microplate reader (such as Molecular Devices: M and i series) which with can measure the absorbance at 450 nm (also with the reference wavelength at 630nm).

2. Assay method

1) Reagent preparation

All reagent components and samples to be tested need be balanced to the room temperature before use and be prepared freshly for use.

a. Preparation of 1×Dilution&Wash Buffer:

Equilibrate the concentrate to room temperature and dissolve well without crystallization. After mixing, dilute Dilution&Wash Buffer (20×), i.e., Concentrate (20×), by 20 times with distilled water at 1:20 as required to end up with 1× Dilution&Wash Buffer. e.g., take 10 mL of Dilution&Wash Buffer (20×) and add it to 190 mL of distilled water. For example, take 10 mL of Dilution&Wash Buffer (20×) and add 190 mL of distilled water to 200 mL of 1× Dilution&Wash Buffer.

*If crystals appear in the Dilution&Wash Buffer (20×) concentrate, warm bath at 50°C in a water bath until the crystals disappear completely.

b. Preparation of Detection Antibody:

The Detection Antibody (1250×) was first removed and placed on an ice box, and then the Detection Antibody, i.e., HRP-conjugated Antibodies (1250×), was diluted 1250-fold with 1×Dilution&Wash Buffer at a ratio of 1:1250, and

finally, we obtained 1× Detection Antibody.

c. Preparation of TMB color solution:

Mix the two color developing substrates, TMB Substrate A and TMB Substrate B, in equal volume (if the color developing solution has turned blue after mixing, please do not use it), mix well and leave it away from light. Usually 10min before use, avoid leaving it for too long, which will affect the effect of use.

2) Standard curve preparation:

Prepare 8 clean 1.5 mL centrifuge tubes and label them sequentially according to the standard concentration.

990 μ L of **1× Dilution&Wash Buffer** was added to the first centrifuge tube and 300 μ L of **1× Dilution&Wash Buffer** was added to each of the remaining centrifuge tubes. Take 10 μ L of the dissolved and mixed 5 μ g/mL standard, add it to the first centrifuge tube, mix it well and label it as 50 ng/mL, then take 300 μ L to the next centrifuge tube labeled with the concentration, mix it well and carry out a series of 2-fold gradient dilutions, with the highest concentration labeled as 50 ng/mL at the beginning and the lowest as 1.5625 ng/mL, which can be formulated according to the following preparation method to proceed. The standard curve should be prepared for each test, and the standard curves of different kits and different times should not be mixed. When testing samples, the amount of standard required for each well is 100 μ L, note that the preparation volume should be higher than the required volume to avoid under-use.

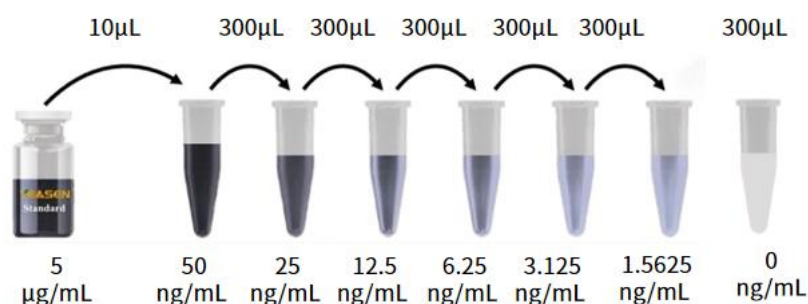


Figure 1 Simplified flow chart of standard configuration

Vial	Volume of 1×Dilution&Wash Buffer (μ L)	Volume of Standard (μ L)	Final concentration of Standard (ng/mL)
A	990	10 (5 μ g/mL)	50
B	300	300 A	25
C	300	300 B	12.5
D	300	300 C	6.25
E	300	300 D	3.125
F	300	300 E	1.5625
G	300	0	0

Table 1 BSA standard preparation

3) Test samples preparation:

a. Dilute the test samples with certain dilution factor by using **1× Dilution&Wash Buffer**. The appropriate dilution of the sample to be tested needs to be mapped. When the concentration of BSA in the sample is unknown, it is generally recommended that the sample be diluted in a gradient of 10-fold, 100-fold, 1,000-fold, etc., and then analyze the content of BSA in the sample by ELISA to determine the appropriate dilution of the sample to be tested.

b. After determining the appropriate dilution of the sample to be tested and the amount of BSA in the sample, the appropriate amount of standard was then added to the sample to be tested to assess the sample spiking recovery.

4) Experiment flow

All reagent components and samples to be tested need be balanced to the room temperature before use. It is strongly recommended that all standards and test samples be assayed in duplicate.

a. Reagents and samples preparation:

Prepare required reagents, diluted standards and samples.

b. Strip Determination:

Calculate the strips required for test samples and standards, take strips from the foil bag, put the remaining strips back into the foil bag, seal the bag, and store at 2°C ~8°C.

c. ELISA plate washing:

Wash the plate for 3 times with 1 × Dilution&Wash Buffer (300 μL/well), and pat the ELISA plate dry. The plate washing has an important impact on the test results, make sure that no wash solution remains in the plate.

d. Samples incubation:

Add standards and test samples, 100 μL/well, ensure that the sample adding is completed within 15 min, and incubate at 37°C for 1 h.

e. ELISA plate washing:

Discard the liquid in the wells, wash the plate with 1 × Dilution&Wash Buffer (300 μL/well) for 5 times, and pat the ELISA plate dry.

f. HRP-conjugated Antibodies incubation:

Add the pre-diluted 1 × Detection Antibody into the ELISA plate, 100 μL/well, and incubate at 37°C for 1 h.

g. ELISA plate washing:

Discard the liquid in the wells, wash the plate with 1 × Dilution&Wash Buffer (300 μL/well) for 5 times, and pat the ELISA plate dry.

h. Color development:

Return the substrate solution to room temperature 10 min before use, add the substrate mixture TMB (A + B) into the ELISA plate, 100 μL/well, and incubate at 37°C away from light for 20 min.

i. Stopping:

Add stop solution to the plate, 100 μL/well, and shake the plate gently until the color is uniform.

j. Reading:

Read the absorbance at 450 nm/630 nm within 10 min. 450 nm as the detection wavelength, and 630 nm as the reference wavelength.

5) Result Analysis

a. If the OD value of the sample exceeds the maximum peak of the standard curve, the sample should be diluted and re-measured.

b. Standard curve drawing:

The standard curve is drawn with the concentration of the standard as the abscissa and the absorbance value of OD_{450nm-630nm} as the ordinate. A variety of mapping and statistical software can be used to aid in drawing standard curves and calculating unknown sample concentrations. The four-parameter fitting method often has

better curve fitting effect, and other methods such as linear and double-logarithm method may also obtain better fitting results, which need to be analyzed according to specific experimental data. Finally, the residual BSA concentration in the sample will be calculated according to the standard curve and the dilution ratio of the sample.

c. The typical Standard Curve is as follows (the following standard curve is for reference only, and the sample content should be calculated based on the standard curve generated in the same experiment) :

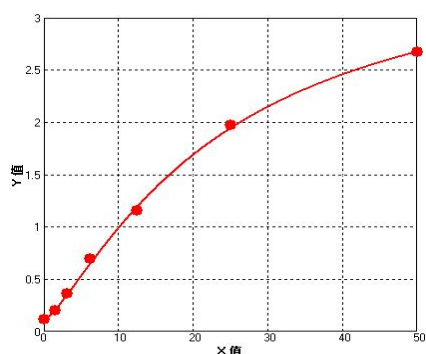


Figure 2 Typical standard curve

Standard Conc. (ng/mL)	OD		Mean OD
50	2.627	2.711	2.669
25	1.918	2.032	1.975
12.5	1.157	1.148	1.153
6.25	0.675	0.717	0.696
3.125	0.376	0.341	0.359
1.5625	0.208	0.199	0.203
0	0.106	0.117	0.111

Table 2 Typical standard curve data

6) Experiment flow chart

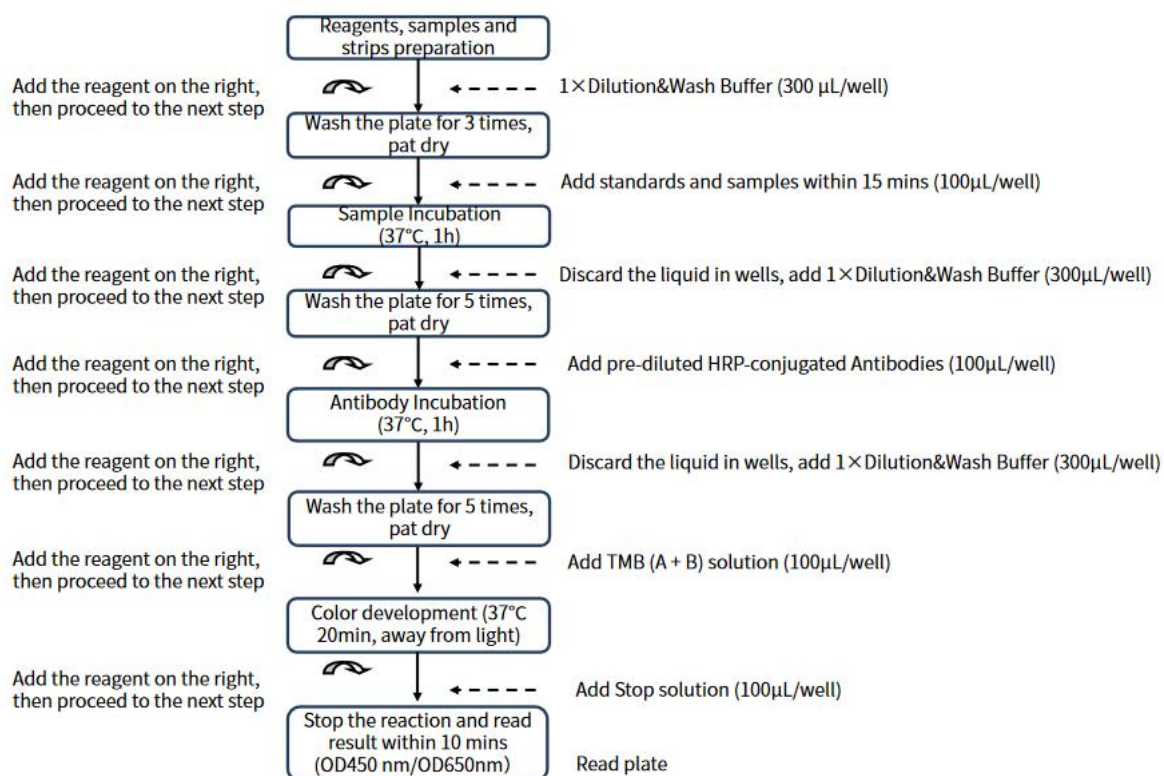


Figure 3 Experiment flow chart

Notes

1. Please read this manual carefully before using this kit. The kit needs to be used up within its shelf life.
2. Mixing of related reagents from different batches is prohibited.
3. The experiment should be conducted in a standardized manner, vortex and mix well for each reagents and samples before use.

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4. The kit is designed for detecting the target antigens and samples marked in the instructions only. Other applications need to be designed and verified by the user, and the reliability and accuracy should be evaluated based on the results.
 5. For your safety and health, please wear personal protective equipment (PPE), such as laboratory coats and disposable gloves, when operating with this product.
 6. This product is for research use only.