BSA ELISA Kit

Catalog Number: S0111B-96

【INTENDED USE】

The kit is used for quantitative detection of BSA in intermediate, semi-finished and finished of various biological products.

【 PRINCIPLE】

This kit using double antibody sandwich enzyme-linked immunosorbent detection technology to determinate residues of BSA in the samples. The capture antibody is coated with 96-well labeling plate and the solid phase antibody is prepared. Then first add the standard and testing samples, and second put a marker on horseradish peroxidase labeled antibody, forming a solid phase antibody-BSA-labeled antibody sandwich, reaction after washing, then add the chromogenic reaction of the substrate. The substrate under the catalysis of HRP become blue, and under the action of stop solution into the final yellow. The absorbance (OD value) is measured at 450nm, and the content of BSA in the sample to be tested is calculated by the standard curve.

【 PRODUCT PERFORMANCE INDICATORS】

Detection limit: <0.5ng/mL

Quantitative limit: 0.5ng/mL

Linear range: 0.5-32ng/mL

Accuracy (reciprocal recovery rate): 80%-120%

Accuracy (measurement deviation): ≤15%

Repeatability (batch difference): ≤10%

【MATERIALS PROVIDED】

Number	Component	Size	Storage condition
1	Microtiter Plate	96T	2-8℃
2	Standard (5 μg/mL)	300 μL× 1 tube	2-8℃
3	HRP-Conjugate Antibody	40 μL×1 tube	2-8℃
4	Buffer (20×)	30 mL× 1 vial	2-8℃
5	Developing solution A	8 mL× 1 vial	2-8℃ (keep in dark)
6	Developing solution B	8 mL× 1 vial	2-8℃ (keep in dark)
7	Stop Solution	15 mL× 1 vial	2-8℃
8	Microplate Sealers	3	-

【STORAGE】

Unopened Kit store at 2-8℃ and the kit is stable for 12 months.

【REAGENTS/EQUIPMENTS REQUIRED BUT NOT SUPPLIED】

1. Enzyme-labeled instrument, constant temperature oscillator (or constant temperature incubator), auto microplate-washer（ Not recommended for cross-use with other projects Or Direct hand wash), whirlpool oscillator, timer
2. High-precision pipette and disposable suction head (0.5-10 μL, 10-100 μL, 30-300 μL, 100-1000 μL)
3. Sterile deionized water without heat source
4. Absorbent paper, EP tube, disposable gloves

【REAGENT PREPARATION】

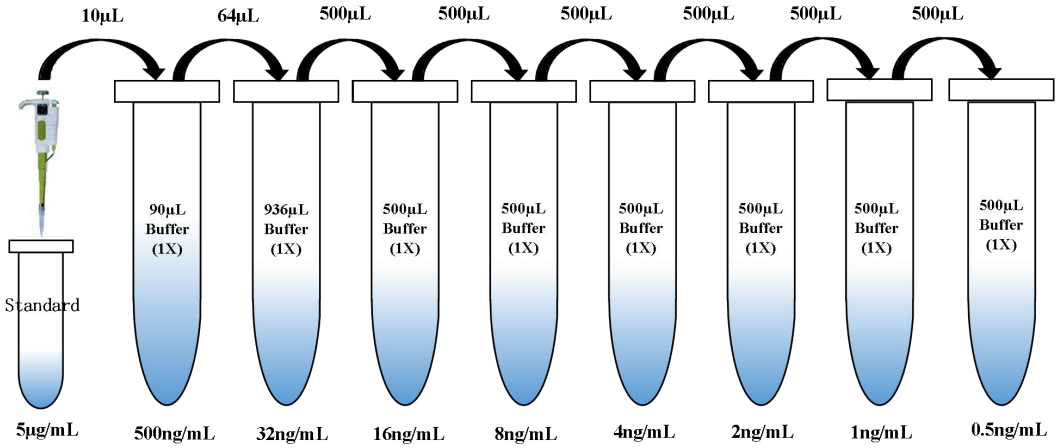
(Note: Try not to weigh BSA powder or place electronic balance that has weighed BSA in the laboratory, clean the experimental table before the experiment, and prepare reagents according to the needs of the experiment)

- Buffer(1×):Take 1 part of Buffer (20×) and add 19 parts of deionized water to prepare a working concentration buffer (1×). If there is crystallization in the buffer (20×), it should be gently shaken at room temperature or 37℃ in a water bath, and then diluted after the crystallization is completely dissolved. Unused buffer (20×) should be stored at 2-8℃.
- Substrate Solution: Mix the developing solution A and developing solution B with equal volume before 10 minutes using. Avoid light to ensure that the substrate solution is not contaminated. If the substrate solution after mixing has turned blue, do not use it.
- Preparation of HRP-Conjugate Antibody solution (1×): Remove the HRP-Conjugate antibody and place it on the ice box, for experimental purposes (100 μL/ well), the HRP-Conjugate antibody was diluted 500 times with Buffer (1×) to become HRP-Conjugate antibody (1×). (Note: HRP-Conjugate antibodies should not be kept at room temperature for a long time, it is best to take them out when needed and operate them on the ice box; Enzyme-labeled antibody (1×) needs to be ready for use).
- Standard preparation: 500 ng/mL standard is prepared by adding 10 μL of 5 μg/mL standard to 90 μL Buffer (1×), then add 64 μL of 500 ng/mL standard to 936 μL Buffer (1×), dilute it to 32 ng/mL, and then dilute it according to 2 times the ratio.

【ASSAY PROCEDURE】

Bring all reagents and samples to room temperature before use.

1. Bring the test slats from the aluminum foil bag that has been balanced to room temperature, put the remaining slats back into the aluminum foil bag, seal them, and store them in 2-8℃ .
2. Preparation of the standard curve: dilute the Standard with Sample dilution to 32 ng/mL, then prepared by Multiple Dilution method.



3. Sample incubation: Add the diluted standard product and the sample to be tested into the enzyme label plate (suggested addition sequence: standard product hole, blank hole, sample hole, standard product according to the concentration gradient), 100μL/ hole; Then each well was added with HRP-conjugate antibody (1×), 100μL/ well, sealed with sealing plate membrane, and incubated at 37℃ and 400-600 rpm for 1h. (Note: The sampling time should be controlled within 10min to avoid drift over time. If the plate is not sealed or the plate is not completely sealed during warming, the reaction liquid will evaporate, resulting in errors in the experiment.)

4. Washing board: discard the liquid in the well and wash the board with buffer (1×) for 3 times (250μL /well). Pat dry residual liquid in sample well. (Before discarding the liquid in the well each time, if washing plate by hand, the liquid can be left for 1min after adding and slightly shaken; If plate washing machine is used, 5s can be slightly shaken after adding buffer).

5. Color development: the pre-prepared substrate solution was added to the enzyme label plate, mixed well (100 μL/well), and incubated in 37℃ for 20 min.

6. Termination: add stop solution (100 μL /well).

7. Recording: The OD value at 450 nm/630 nm should be measured by placing the plate in the microcoder, and the reading should be completed within 20min.

【CALCULATION OF RESULTS】

1. Calculation of absorbance value: The calibrated value of light absorbance of each standard or sample is: ($OD_{450\ nm}$-$OD_{630\ nm}$)- absorbance valueof the blank control well ($OD_{450\ nm}$-$OD_{630\ nm}$) .

2. Plot the standard curve with concentration of the standard as the horizontal coordinate (X) and the calibrated value of light absorption of the standard as the vertical coordinate (Y), and a four -parameter Logistic mathematical model is recommended to fit the equation:Y=（（A-D）/（1+

（X/C）^B））+D.Substitute OD value of the sample ($OD_{450\ nm}$ - $OD_{630\ nm}$) - absorbance value of the blank control well($OD_{450\ nm}$-$OD_{630\ nm}$)) into the formula to calculate the content of BSA in the sample, and note the dilution factor.

3. If the OD value of the sample to be tested exceeds the highest OD value of the standard curve, the sample shall be redetermined after dilution.

【PRECAUTIONS】

1. The container for measuring BSA content should be dedicated to prevent BSA contamination in the laboratory.
2. The background value of this kit is generally not greater than 0.2. If the background value is greater than 0.2, it is necessary to check the environment, utensils, consumables, etc., and you can also use unoped bottled Wahaha water for water quality check.
3. All components in the kit must be returned to room temperature (20-25 ° C) before use.
4. Each component should be thoroughly mixed before use to ensure the uniformity of the reagent. The standard product should also be centrifuged for a short time for 5s, concentrate all the liquid on the tube wall and lid at the bottom of the tube, and immediately put all the reagents back to 2-8℃ after use.
5. The kit must be used within the validity period, and the corresponding standard curve must be prepared for each test. It is not recommended to use different batches of related reagents in mixed batches.
6. Be careful not to touch the bottom of the microplate when adding liquid to the enzyme label plate to prevent damage to the coating layer. To avoid cross contamination, replace the loading tank and suction head in time between different samples and steps.
7. When the slats are washed and patted dry, pay attention to prevent the slats from falling off, and the sealing plate film cannot be reused.
8. When the residual concentration of the target to be measured is too high, black flocculent may be produced during color development. In this case, the sample needs to be diluted to a certain extent before detection.
9. When reading, pay attention to check whether the detection wavelength and fitting equation are selected correctly.
10. Only by strictly following the operating methods of the instructions, all the reagents of this kit can be used to ensure the best detection results.
11. The difference in test results can be caused by a variety of factors, including the operation of the experimentor, the way the pipette is used, the washing technique, the reaction time or temperature, and the storage of the kit.

12. The company is only responsible for the kit itself, not for the sample consumption caused by the use of the kit. Please fully consider the possible use of the sample before use, and reserve sufficient samples.

13. This kit is only used for in study, not for clinical diagnosis.

【SAFETY INSTRUCTIONS】

1. All biological materials should be handled and discarded as potentially hazardous following local laws and regulations.

2. Personal protective equipment such as lab coats, gloves, surgical masks and goggles are necessary in experiments for safety reasons.

【PROBLEM ANALYSIS】

If there is any problem with the experimental results, please take photos of the color rendering results in time, and properly preserve the unused slats and reagents. Then contact technical support to find out the reason.

Problem Description	Possible Reasons	Solution
Gradient difference of standard curve	Dilution error	The standard curve is not diluted according to the dilution factor
	The liquids are aspirated or added inaccurately	Check the pipette and tips
	The ELISA plates are not washed completely	Guarantee the plate washing times, and the amount used for washing liquid per well
The color development is weak or colorless	The incubation time is too short	Guarantee sufficient incubation time
	The experiment temperature is not correct	Use the recommended incubation temperature
	The reagent volume is insufficient or miss to add the reagent	Check the liquid aspiration and adding process, and guarantee all the reagents are added sufficiently in sequence
	The developing solution are prepared wrongly	The developing solutions A and B are mixed in same volume and protected from light, which are prepared 10 min before color development
The OD value are of low reading	The sample is not treated	The samples are tested after centrifugation by heating
	The plate reader are set incorrectly	Check the wavelength and optical filter device on the plate reader

		The plate reader shall be opened in advance before reading for preheating
The OD value are of high reading	The sample is not diluted	It is recommended to validate the dilution factor of the sample
The coefficient of variation (CV value) is large	The liquid adding is incorrect	Check the liquid adding condition
	There are pollution at the bottom of the ELISA plate	Check the bottom of the ELISA plate for residual liquid or handprint
	There are foreign matters or bubbles in the plate well	Confirm that there are no foreign matters in the plate well before adding samples, and confirm that there are no bubbles after adding samples
	The plate is not sealed or sealed incompletely during incubation	Seal the plate with a plate sealer
The background value is high	The ELISA plates are not washed completely	Wash the plate according to the method recommended in the instruction for use
		If automatic plate washer is used, please check all the liquid adding port and waste liquid outlet for blocking
		If the plate is washed by hands, the plate washing times can be added properly
		Insufficient washing or missing to wash may lead to high background
	Incubation time and temperature	Operate in strict accordance with requirements in the instruction for use
	Consumption pollution	The consumables used (such as tubes, tips etc.) are not clean
	The washing solution is polluted	Prepare fresh washing solution
	The developing solution is polluted	The color developing solution itself does not have colors, please ensure that the color developing solution is not polluted by metal ion or oxidizing agent before use, and it is protected from light.
The sensitivity is low	The kit is stored improperly	Store the related reagents in accordance with the requirements in the instruction for use

The specification is issued in version A/1 with an effective date of 2025/08/13.

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