Instructions for Use of BSA ELISA Detection Kit

The kit is intended for scientific research only and should not be used for diagnosis

Cat. No. HG- BS001

Introduction

The BSA ELISA Detection Kit is designed for the quantitative detection of residual BSA content in intermediates, semi-finished products and finished products of various biological products

The kit can quantitative detect residual BSA content in test samples by using a double-antibody sandwich method. Coat the 96-well plate with capture antibody to prepare immobilized antibodies. Add the standard and test samples, and then horseradish peroxidase (HRP) conjugated antibody to form an immobilized antibody - BSA - enzyme-labeled antibody sandwich conjugate. Wash the plate after reaction, and add the substrate for color development reaction. The substrate will turn blue under HRP catalysis, and will finally turn yellow under the action of the stop solution. Determine the optical density (OD) value at 450 nm, and calculate the BSA content in the test sample using the standard curve.

Specification

96 T

Kit components

Components	Specification	Preparation
BSA Coated Plate	8 wells × 12 strips	Ready-to-use
BSA standard (standard)	100 μL × 1 vial (5 μg/mL)	Operate as per the recommended dilution procedure
1250× Anti-BSA (1250× enzyme-labeled antibody)	30 μL × 1 vial	Operate as per the recommended dilution procedure
20 × Buffer	30 mL× 1 bottle	Operate as per the recommended dilution procedure
Color Reagent A	8 mL× 1 vial	Ready-to-use
Color Reagent B	8 mL× 1 vial	Ready-to-use
Stop Solution	15 mL× 1 bottle	Ready-to-use
Plate Sealer	3 pieces	Ready-to-use
Instructions for Use	1 copy	/

Notes: Reagents should be stored at 2 ~ 8℃; Color Reagents A and B should be protected from light during storage.

Storage conditions and shelf life

The shelf life of unopened kits is 12 months when stored at 2 ~ 8℃.

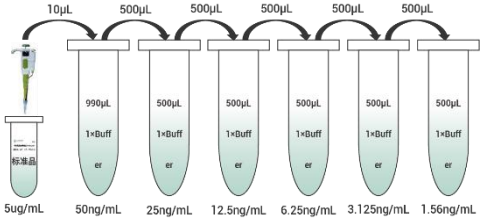
Apparatus to be prepared by the user:

1. Plate reader, thermostate plate shaker or thermostate incubator, plate washer (should not be cross-used in other projects).
2. High-precision pipette and disposable tips (0.5 ~ 10 μL, 10 ~ 100 μL, 30 ~ 300 μL, and 100 ~ 1000 μL).
3. Deionized water, blotting paper, EP tubes

Reagent preparation

1. Avoid placing electronic balances or weighing BSA powder in the laboratory as much as possible. Clean the benchtop before experiments.

2. Preparation of 1× Buffer: Take 1 portion of Buffer (20×), and add 19 times the volume of deionized water to prepare the working concentration buffer (1×). If there are crystals in the Buffer (20×), shake gently at room temperature or in a 37℃ water bath, and dilute after the crystals are completely dissolved. Unused Buffer (20×) should be stored at 2 ~ 8℃.



Notes: Use clean, dedicated containers to prepare the Buffer as needed, and do not share solutions, containers, or consumables with other projects.

3. Preparation of standard: Dilute the standard to 50 ng/mL with 1× Buffer, and prepare the standard by 2-fold dilution, as shown in the figure above.

4. Preparation of 1× enzyme-labeled antibody: Take out the 1250× enzyme-labeled antibody from the freezer and place it on an ice box. Dilute the 1250× enzyme-labeled antibody with 1× Buffer by 1250 folds based on the volume required (100 μL/well) to prepare 1× enzyme-labeled antibody. Do not leave the 1250× enzyme-labeled antibody at room temperature for a long time. It is preferred to take the antibody out when needed and perform operations on an icebox. The 1× enzyme-labeled antibody should be prepared freshly before use.

5. Preparation of substrate solution: Mix Color Reagents A and B at equal volume at 10 minutes before use, and the operation should be performed at dark environment. Make sure that the substrate solution is not contaminated. Do not use if the substrate solution turns blue after mixing.

Test procedures

1. Equilibrate the temperature of each component in the kit to room temperature for 30 minutes. Take out required strip plates from aluminum foil bags already equilibrated to room temperature, and label the strip plate sequence with a marker. Seal remaining strip plates with a plate sealer, put them back to the aluminum foil bag, then seal the bag, and store at 2 ~ 8℃.

2. Sample incubation: Set the standard wells, blank wells, and test sample wells, respectively. Add standards at different concentrations (in sequence), 1× Buffer, and test sample to standard wells, blank wells, and test sample wells, respectively (100 μL/well; replicate well is recommended to avoid drifts), seal the plate with a plate sealer, and incubate at 37℃ for 1 hour.

3. Plate washing: Discard liquid in the wells; wash the plate for 3 times with 1× Buffer (300 μL/well), and pat dry the residual liquid in wells on a blotting paper. (If the plate is washed manually, avoid the pipette tip touching the inner wall of each well when adding 1× Buffer for plate washing. After adding Buffer each time, allow to stand for 1 minute and shake gently. When patting for drying, use a new blotting paper each time, or dry the plate in a clean area of the paper)

4. Incubation of enzyme-labeled antibody: Add 1× enzyme-labeled antibody at 100 μL/well, seal the plate with a plate sealer, and incubate at 37℃ for 1 hour.

5. Plate washing: Same as Step 3; wash the plate for 5 times.

6. Color development: Add the pre-prepared substrate solution into the plate at 100 μL/well, seal the plate with a plate sealer, and allow the solution to stand and incubate at 37℃ for 20 minutes while being protected from light.

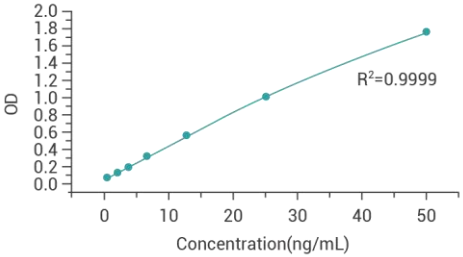
7. Reaction termination: Add stop solution at 100 μL/well.

8. Reading: Measure the OD values at 450 nm and 630 nm with a plate reader. The measurement should be completed within 20 minutes after reaction termination

Results process

Plot the standard curve with the OD value ($OD_{450\,nm}$ - $OD_{630\,nm}$) of the BSA standard as the dependent variable Y and the standard concentration (ng/mL) as the independent variable X. The 4-parameter logistic fitting equation is recommended: Y = ((A - D)/(1 + (x/C)^B)) + D. Substitute the OD value of test sample ($OD_{450\,nm}$ - $OD_{630\,nm}$) to the equation of the standard curve to calculate the BSA content in test sample.

Standard curve



Assay result

Standard concentration (ng/mL)	OD value (1)	OD value (2)	Mean value
50	1.775	1.803	1.789
25	1.091	0.995	1.043
12.5	0.571	0.591	0.581
6.25	0.324	0.308	0.316
3.125	0.193	0.179	0.186
1.56	0.132	0.127	0.130
0	0.072	0.088	0.080

Precautions

1. The containers used for BSA assay should be dedicated, so as to prevent BSA contamination in the laboratory.
2. This kit is for *in vitro* detection only, and may not be used for clinical diagnosis.
3. The kit must be used within the shelf life. Mix well each component before use.
4. To ensure accurate assay, please strictly follow the instructions for use, use only the reagents provided in the kit, and do not use self-prepared reagents or reagents from other kits.
5. Please timely replace reagent troughs and pipette tips when loading different samples and performing different steps,

so as to avoid cross contamination.

6. The final assay results are closely related to reagent effectiveness, the operations of analysts, and the test environment.

7. Our company is only responsible for the kits themselves, and will not be responsible for the sample consumption caused by kits during use. Users should fully consider the possible sample consumption before operation, and should reserve sufficient sample size.

8. The stop solution in this kit is acidic, thus extra attention should be paid during operation.

9. All biological samples may cause potential biosafety risks, therefore, users must strictly follow local laws and regulations when handling and disposing of the samples.

Problem analysis

In case of any problems with the assay results, please take photos of the color development results in a timely manner, properly store the unused strip plates and reagents, and contact us for technical support. Alternatively, the user may refer to the following information to identify the cause.

Problem description	Possible reason	Corresponding measure
Poor gradient of standard curve	Inaccurate liquid pipetting or addition	Check the pipette and tips
	Insufficient plate washing	Ensure the number of plate washing times and the volume of Wash Buffers for each well
Very weak or no color	Excessively short incubation duration	Ensure adequate incubation duration
	Incorrect experimental temperature	Adopt the recommended incubation temperature
	Inadequate reagent volume or missed addition of reagent	Check procedures of liquid pipetting and addition, so as to ensure that all reagents are added in specified order and at adequate volume.
Low OD value	Incorrect setting of plate reader	Check the wavelength and filter device on the plate reader
		Preheat the plate reader in advance before reading
Large CV	Improper liquid addition	Check procedure of liquid addition
	Contaminations at plate bottom	Check the plate bottom for any remaining liquid or fingerprints
	Foreign matters or air bubbles in the wells	Make sure there are no foreign matters in the wells before loading, and make sure there are no air bubbles in the wells after loading
High background value	Insufficient plate washing	Wash the plate as per the method recommended in the instructions for use
		If using an automatic plate washer, please check all the liquid addition ports and discharging outlets for blockages
		If washing the plate manually, increase the number of plate washing times as appropriate.
	Contaminated Wash Buffer	Prepare fresh Wash Buffer
Low sensitivity	Improper storage of the kit	Store relevant reagents as per the instructions for use.

Disclaimer

Under all circumstances, the liability of our company for this product is only limited to the value of the product itself.

Jiangsu Hillgene Biopharma Co., Ltd.
Address: Building 4, Yuewang Wisdom Valley, 1463 Wuzhong Avenue, Wuzhong District, Suzhou, China
T.400 900 1882 W.www.hillgene.com