SHENTEK

Bovine Serum Albumin (BSA)
ELISA Kit
User Guide

PLEASE READ THE DOCUMENT CAREFULLY BEFORE EXPERIMENT

Product No.: 1401401
Version: A/0
For Research Use Only

Huzhou Shenke Biotechnology Co., Ltd

Product Name

Bovine Serum Albumin (BSA) ELISA Kit

Package

96 tests/Kit

Intended Use

This kit is suitable for the quantitation of residual bovine serum albumin (BSA) from in-process to end product.

The kit is for RESEARCH USE ONLY and not intended for clinical use.

Product Description

This kit is based on the solid-phase enzyme-linked immunosorbent assay (ELISA) with a double-antibody sandwich technique to detect residual bovine serum albumin (BSA). A sheep polyclonal antibody specific to BSA was employed in the assay to capture any remaining BSA impurities in the sample. Both the Calibration standards and test samples were simultaneously added to the microtiter plate coated with the affinity purified capture antibody, and followed by incubation and washing. The biotinylated antibody was added to the microtiter plate to bind the BSA and then reacted with streptavidin labeled HRP (Horseradish Peroxidase). TMB (3,3',5,5' -tetramethylbenzidine) substrate was added into reaction, HRP catalyzed the oxidation of TMB by H_2O_2 to produce a blue product (maximum absorption peak at 655 nm). Then the stop solution was added to terminate the enzymatic reaction, resulting in a yellow colored product (maximum absorption peak at 450 nm). The absorbance values at 450 nm wavelength were positively correlated with the BSA concentration in the calibration standard and the samples. The concentration of BSA in the samples can be calculated using a dose-response curve.

■ Kit Contents

Table 1.Kit Components

Reagent	Part No.	Quantity	Note
BSA Calibration standard	PNB007	1 × 500 µL	Please refer to the details on the label of the tube.
Anti-BSA Microtiter Strips	PNA007	8 well × 12 strips	Strips pre-coated with sheep anti-BSA antibody in a vacuumed bag with desiccant. Seal and store immediately after use.
Wash Buffer Concentrate (10×)	PNF001	2 × 25 mL	For plate washing and dilution of calibration standards, biotinylated antibody, streptavidin-HRP and samples. Dilute 10 times in freshly prepared ultra-pure water to obtain 1×Wash Buffer solution.
Anti-BSA : Biotinylated Conjugate (100×)	PNG007	1 × 120 µL	Biotinylated anti-BSA antibody (sheep polyclonal) in a protein matrix with preservative. Sealed and keep away from light. Dilute 100 times in 1×wash buffer before use.
Streptavidin-HRP (100×)	PNH002	1 × 140 µL	Streptavidin labeled with HRP. Sealed and keep away from light. Dilute 100 times in 1×wash buffer before use.
TMB Substrate	PND005	1 × 12 mL	3,3',5,5'-tetramethylbenzidine；Sealed and keep away from light. Equilibrate to room temperature (RT) for 20 minutes before use.
Stop Solution	PNI002	1 × 6 mL	1 M hydrochloric acid. Avoid direct contact with eyes, skin, and clothing. Wear goggles while handling.
Sealing Film	PNK001	3 pieces	Cover the strips with it during incubation to prevent contamination and liquid evaporation.

Note: Room temperature refers to 25 ± 3℃.

■ Storage Conditions

Store the kit at 2-8℃. Please check the expiration date on the labels. The opened components should be stored as shown in Table 2.

Table 2. Recommended storage conditions for opened components

Component	Stability
Anti-BSA microtiter strips	Store in the bag with desiccant at 2-8℃ for up to 30 days.
BSA Calibration standard	Store at 2-8℃ for up to 1 year.

■ Materials Required But Not Provided

- Sterile centrifuge tubes for dilution
- Absorbent paper for plate drying
- Pipette Tips: 1000 μL, 100 μL and 10 μL
- Multi-channel reagent reservoirs (50 mL)

■ Equipment

- Microplate reader capable of measuring absorbance at 450 nm, with the correction wavelength set at 620 nm to 650 nm.
- Single or multi-channel micropipettes.
- Vortex mixer
- Incubator (optional)
- Plate washer (optional)
- Water bath (optional)

■ Workflow

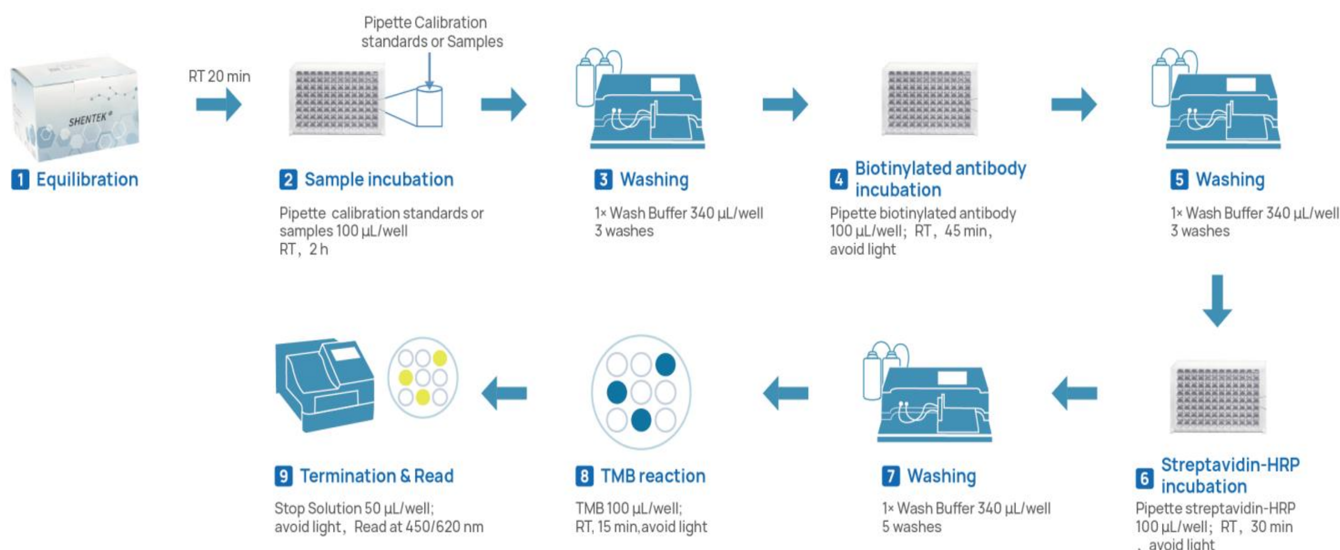


Figure 1. Procedure Flowchart

1. Preparation

(1) Equilibration

- Before use, allow the kit to equilibrate at room temperature for 20 minutes, return to 2-8°C immediately after use.
- Take appropriate amount of strips to a strip holder according to the experiment design. Please store the remaining strips in the bag with desiccant at 2-8°C.

(2) Preparation of Reagents

- 1×Wash Buffer: Dilute 1 volume of Wash Buffer with 9 volumes of ultra-pure water. For example, add 25 mL Wash Buffer Concentrate (10×) to 225 mL of ultra-pure water to make 250 mL of 1×Wash Buffer. Mix well before use.

  Note: If the Wash Buffer Concentrate (10×) or Diluent is cloudy or contains precipitates, heat at 37 °C in the water bath until it clears.

(3) Preparation of Calibration standard Solutions

- Prepare BSA Calibration standard solutions as indicated in Fig 2 and Table 3.

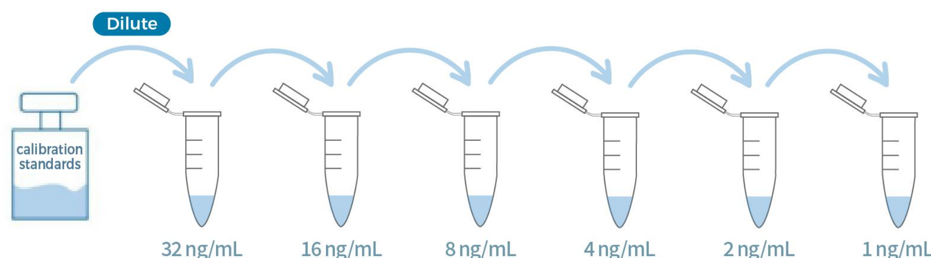


Figure 2. Graphic scheme of BSA Calibration standard solutions

Table 3. Preparation of BSA Calibration standard solutions

Serial Dilution Tube	Dilution procedure	Conc.(ng/mL)
ST1	Dilute the BSA Calibration standard to ST1 with 1×Wash Buffer	32
ST2	500 μL ST1 + 500 μL 1×Wash Buffer	16
ST3	500 μL ST2 + 500 μL 1×Wash Buffer	8
ST4	500 μL ST3 + 500 μL 1×Wash Buffer	4
ST5	500 μL ST4 + 500 μL 1×Wash Buffer	2
ST6	500 μL ST5 + 500 μL 1×Wash Buffer	1
NCS	1×Wash Buffer	0

(4) Sample Preparation

- Test samples: In-process samples, harvested bulk, drug substance and drug product. Make sure samples are clear and transparent, and insoluble substances need to be removed by centrifugation or filtration.
- Conduct sample stability studies to prevent degradation or denaturation during the experiment. Avoid repeated freeze-thraw cycles. For long-term storage, -70°C or below is recommended to avoid degradation.
- Dilute the samples with 1×Wash Buffer to achieve a proper range of BSA concentration within the calibration curve.
- For the first use, a method validation is recommend to verify sample suitability before the subsequent routine test. This will help to set up appropriate sample dilution series.

Note: Please contact us for support of validation protocol.

2. Assay Experiment

(1) Sample Incubation

- Pipette 100 μL of Calibration standard, controls and samples into each designated well according to the experimental design. Avoiding foaming bubbles during pipetting. It is recommended to prepare 2-3 replicates for each concentration.
- Seal the plate and incubate for 2 hours at room temperature.

Table 4. Example of the 96 tests layout

	1	2	3	4	5	6	7	8	9	10	11	12
A	NCS	NCS	NCS		S1	S1	S1					
B					S2	S2	S2					
C	ST6	ST6	ST6		S3	S3	S3					
D	ST5	ST5	ST5		S1+SRC	S1+SRC	S1+SRC					
E	ST4	ST4	ST4		S2+SRC	S2+SRC	S2+SRC					
F	ST3	ST3	ST3		S3+SRC	S3+SRC	S3+SRC					
G	ST2	ST2	ST2									
H	ST1	ST1	ST1									

✧ "ST1-ST6" indicate 6 concentration gradients, "NCS" as negative control, "S1-S3" as test samples , and "S1 SRC-S3 SRC" as the spiked recovery controls for each sample.

✧ The number of replicates and the spiked samples can be determined by method validation.

(2) Biotinylated Antibody Reagent Preparation and Incubation

- 1×Anti-BSA:Biotinylated Conjugate: Prepare the 1×Anti-BSA:Biotinylated Conjugate by diluting the Anti-BSA: Biotinylated Conjugate (100×) with 1× Wash Buffer in a new centrifuge tube. Prepare 1×Anti-BSA:Biotinylated Conjugate fresh, mix gently and use it immediately.
- Wash the plate with 340 μL of 1×Wash Buffer per well and soak for 30 seconds. Wipe off any liquid from the bottom outside of the plate. Repeat washing for 3 times. Do not allow the wells to be completely dry before adding the substrate.
- Pipette 100 μL of 1×Anti-BSA:Biotinylated Conjugate into the corresponding wells as indicated earlier.
- Seal the plate and incubate for 45 min at room temperature, and protect from

light.

(3) Streptavidin-HRP Preparation and Incubation

- 1×Streptavidin-HRP: Prepare the 1×Streptavidin-HRP by diluting the Streptavidin-HRP (100×) with 1×Wash Buffer in a sterile centrifuge tube. Prepare 1×Streptavidin-HRP fresh, mix gently and use it immediately.
- Wash the plate with 340 µL of 1×Wash Buffer per well and soak for 30 seconds. Wipe off any liquid from the bottom outside of the plate. Repeat washing for 3 times. Do not allow the wells to be completely dry before adding the substrate.
- Pipette 100 µL of 1×Streptavidin-HRP into the corresponding wells.
- Seal the plate and incubate for 30 min at room temperature, and protect from light.

(4) TMB Reaction

- Equilibrate the TMB substrate for 20 min at room temperature.
- Wash the plate with 340 µL of 1×Wash Buffer per well and soak for 30 seconds. Wipe off any liquid from the bottom outside of the plate. Repeat washing for 5 times. Do not allow the wells to be completely dried before adding the substrate.
- Add 100 µL of TMB Substrate into the wells, and incubate at room temperature for 15 min, and protect from light.

  Note : Do not use sealing film during this step.

(5) Termination

- Add 50 µL of Stop Solution into each well.

  Note: The order of adding stop solution should be the same as the order of adding the TMB substrate. While adding samples, suspend the tips above the liquid to prevent contact with the solution in the wells and minimize the risk of bubble formation.

(6) Reading

- Read absorbance at 450 nm/620-650 nm.

3. Calculation and Analysis

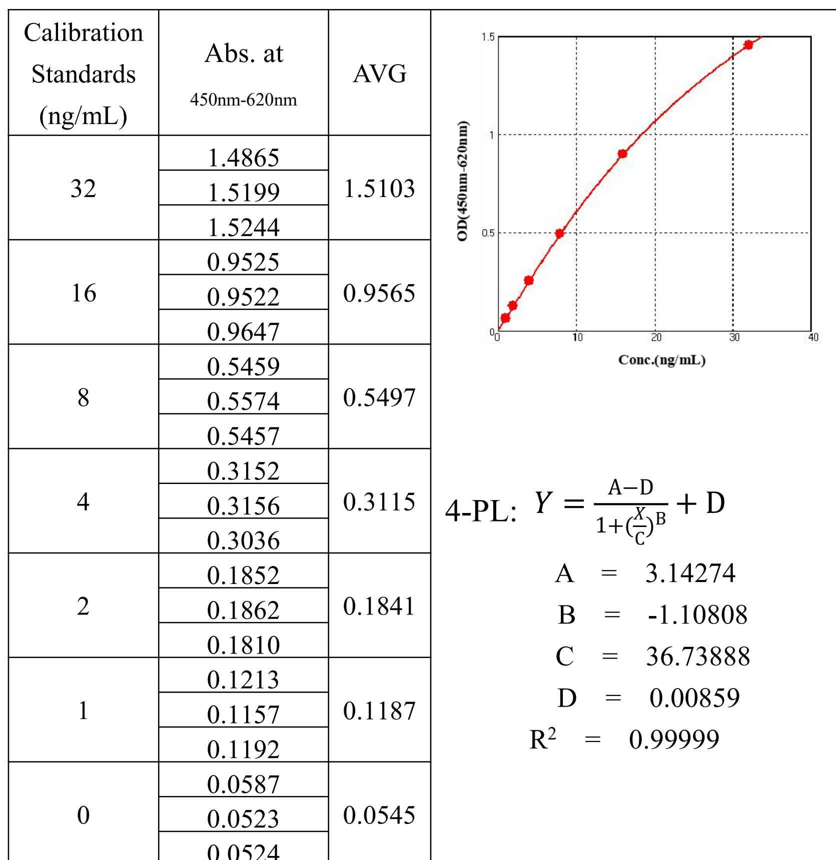
- The OD value of each well should be calculated by the difference between OD_{450nm} and their respective long wavelength. If the microplate reader is not equipped with long wavelength measurement, this step can be omitted.
- Subtract the OD value of the NCS from each calibration point and samples, and record the mean of the replicate wells.
- Perform a 4-parameter logistic regression model using the Calibration standard concentration values and OD values to obtain the calibration curve equation. Substitute the average OD value of the sample into the equation to calculate the sample concentration, which should be multiplied by the dilution factor to obtain the actual sample concentration.
- The software for data analysis of the standard curve could be the one that comes with the microplate reader. If not, we recommend to use professional standard curve software such as Curve Expert, ELISA Calc, and so on.
- For samples with absorbance values above the Calibration standard ST1, a pilot study should be performed to determine an appropriate dilution before retesting. The BSA concentration in the sample is calculated from the test value multiplied by its corresponding dilution factor. If the spiked samples are simultaneously set at this dilution level and the recovery rate should meet the requirements of the corresponding regulations.

■ Limitations

- This product is intended for research use only but not for clinical use.
- It is recommended to keep the pH of the sample solution between 6.5 and 8.5, as exceeding this pH range may affect the results of the sample assay.

■ Assay Performance

- Linearity& Range: 1-32 ng/mL， $R^2 \geq 0.990$
- LLOQ: 1 ng/mL
- Specificity: Cross-reactivity rates with host cell proteins (e.g. HEK293T, MDCK, CHO, BL21 and Vero cells) are less than 0.1%.
- Typical calibration curve and data:



Calibration Standards (ng/mL)	Abs. at 450nm-620nm	AVG
32	1.4865	1.5103
	1.5199	
	1.5244	
16	0.9525	0.9565
	0.9522	
	0.9647	
8	0.5459	0.5497
	0.5574	
	0.5457	
4	0.3152	0.3115
	0.3156	
	0.3036	
2	0.1852	0.1841
	0.1862	
	0.1810	
1	0.1213	0.1187
	0.1157	
	0.1192	
0	0.0587	0.0545
	0.0523	
	0.0524	

4-PL: $Y = \frac{A-D}{1+(\frac{X}{C})^{B}} + D$

A = 3.14274

B = -1.10808

C = 36.73888

D = 0.00859

R^2 = 0.99999

Additional Information

- ✧ This kit is intended for use by qualified technicians only.
- ✧ Perform the assay in an environment free from BSA contamination.
- ✧ Consumables, for example sterile disposable tips, tubes and reservoirs are only allowed for single use. It is recommended to wipe with 75% ethanol before and after each use. Follow the specified pipetting procedure carefully.
- ✧ Users should validate the assay before testing their samples.
- ✧ Dilution should be gentle and thorough to avoid excessive foaming.
- ✧ Stop Solution is 1M HCl. Avoid direct contact with eyes, skin, and clothing.
- ✧ Do not mix the kit reagents from different lot numbers .
- ✧ Use fresh sterile water or ultra-pure water, and ensure the water temperature does not exceed 37°C.
- ✧ Seal or cover the microplate immediately after sample incubation to avoid liquid evaporation.
- ✧ Avoid drying the wells before TMB substrate incubation.
- ✧ Store unused microtiter strips in a sealed bag with desiccant to prevent contamination.
- ✧ Centrifuge Anti-BSA: Biotinylated Conjugate (100×)and Streptavidin-HRP (100×) before use to avoid any loss of the reagent.
- ✧ To avoid pipetting errors, pipette or sampling accurately for dilution of standards and samples, for example, a minimum volume of 5 µL is recommended.
- ✧ BSA Calibration Standard Solution, Biotinylated Antibody Solution and Streptavidin-HRP Solution are recommended for single use due to stability issue. Prepare freshly before each experiment.
- ✧ TMB Substrate should be colorless. If not, discard it and contact us for assistance.
- ✧ Pipette carefully to avoid any bubbles, and gently shake the plate for thorough mixing. Sometimes air, resulting in bubbles, can be drawn into the micropipette or dispensed into the wells. If this happens, bubbles can influence optical density values and detection results.
- ✧ Plate reading should be completed within 30 minutes after termination.
- ✧ The assay workflow includes 3 washe steps and a manual wash is preferred for the first wash after sample incubation.
- ✧ Avoid the samples containing sodium azide (NaN_3), which will deactivate the HRP and lead to the underestimation of BSA levels.

■ Troubleshooting

Problem	Possible Cause	Solution
High background signal (OD)	Cross-contamination of reagents, including ultra-pure water	Freshly prepared prior to experiment.
	Cross-contamination of equipment, including pipettes and centrifuge	Clean the equipment with 75% ethanol before experiment.
	Environment contamination	Separate the working bench to avoid contamination.
	Insufficient washing	Increase the wash buffer volume or wash times, and remove any remaining liquid before proceeding to the next step.
Abnormal values	Improper washing	Swiftly and completely shake off any excess liquid, and avoid reusing paper towels to minimize contamination.
	Improper sampling	Use the pipette to add the samples to the bottom of the wells and avoid splashing to the neighboring wells.
	Plate sealing	Promptly cover the plate with the sealing film and remove it carefully to prevent splashing.

If you have any other questions, please contact us for technical support.

■ References

- ICH. M10 Bioanalytical Method Validation And Study Sample Analysis
- FDA. Bioanalytical Method Validation Guidance for Industry
- ChP<9012>Guidance of Quantitative Method Validation for Biological Samples
- CHP <3411> Determination of Residual Bovine Serum Albumin

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Support & Contact

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