Human Serum Albumin (HSA) ELISA Kit (One-step ELISA) User Guide

PLEASE READ THE DOCUMENT CAREFULLY BEFORE EXPERIMENT

Product No.: 1402427
Version: A/0
For Research Use Only

Huzhou Shenke Biotechnology Co., Ltd

■ Product Name

Human Serum Albumin (HSA) ELISA Kit (One-step ELISA)

■ Package

96 tests/Kit

■ Intended Use

This kit is suitable for the quantitation of Human Serum Albumin (HSA) from in-process samples to end product.

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

■ Product Description

This kit employs a solid-phase Enzyme-linked Immunosorbent Assay (ELISA) with a double-antibody sandwich technique to detect Human Serum Albumin (HSA) in the sample. Polyclonal antibody specific to HSA was employed in the assay to capture any HSA in the samples. Both the Calibration Standards (or test samples) and the HRP (Horseradish Peroxidase) labeled with anti-HSA antibody were simultaneously added to the pre-coated microtiter plate, and followed by incubation and washing steps. Then 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 colored product (maximum absorption peak at 655 nm). Afterwards 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 HSA concentration in the Calibration Standards and the samples. The concentration of HSA in the samples can be calculated using a dose-response curve.

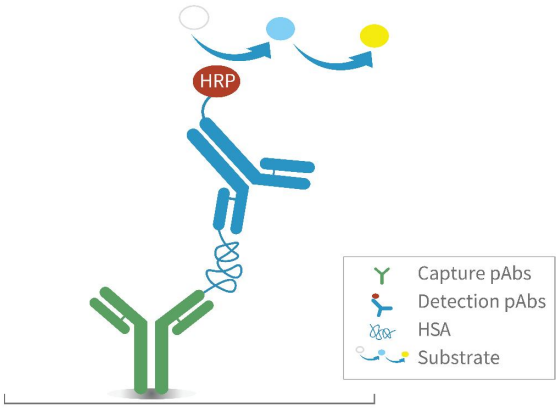


Figure 1. Schematic diagram

■ Kit Contents

Table 1. Kit Components

Reagent	Part No.	Quantity	Note
HSA Calibration Standard	PNB018	1 × 150 µL	Please refer to the details on the label of the tube.
Anti-HSA Microtiter Strips	PNA017	8 well × 12 strips	Strips pre-coated with sheep anti-HSA antibody in a vacuumed bag with desiccant. Seal and store immediately after use.
Diluent	PNE004	2 × 25 mL	For dilution of Calibration Standards, Anti-HSA:HRP (100×) and samples.
Wash Buffer Concentrate (10×)	PNF001	1 × 25 mL	Easy to be crystallize at low temperature, please incubate at 37℃ in water bath before use. Dilute 10 times with freshly prepared ultra-pure water to obtain a 1×Wash Buffer solution for plate washing.
Anti-HSA:HRP (100×)	PNN012	1 × 120 µL	Affinity purified sheep antibody conjugated to HRP. Dilute 100 times in Diluent before use.
TMB Substrate	PND004	1 × 12 mL	Sealed and keep away from light. Equilibrate at room temperature 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 follows.

Table 2. Recommended storage conditions for opened components

Component	Stability
Anti-HSA Microtiter Strips	Store in the bag with desiccant at 2-8℃ for up to 90 days.

■ Materials Required But Not Provided

- Sterile micorcentrifuge 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: 1000 μL, 100 μL, and 10 μL
- Microplate thermoshaker
- Incubator (optional)
- Plate washer (optional)

■ Workflow

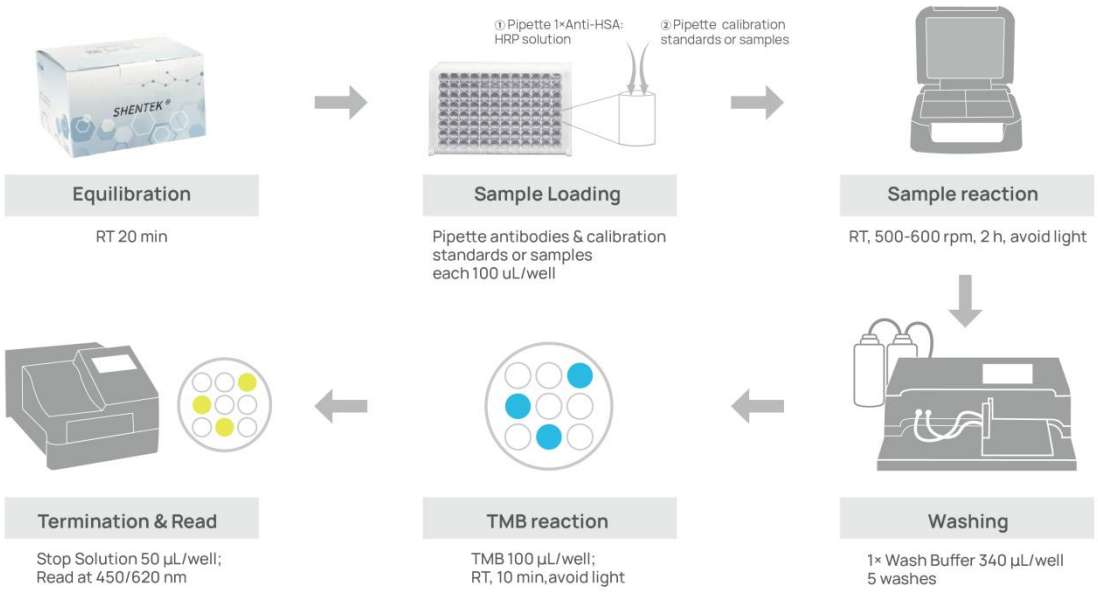


Figure 2. Procedure Flowchart

1. Preparation

(1) Equilibration

- Before use, allow the kit to equilibrate at room temperature for 20 minutes. Return to 2-8℃ after use.
- Take the 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℃.

(2) Preparation of Reagents

- 1×Wash Buffer: Dilute 1 volume of the Wash Buffer Concentrate (10×) 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. Prepare fresh and mix well before use.

  Note: If the Wash Buffer Concentrate (10×) or Diluent is cloudy or contains precipitates, heat at 37℃ until it clears.
- 1×Anti-HSA:HRP: Prepare the 1×Anti-HSA:HRP by diluting the Anti-HSA:HRP (100×) with Diluent in a sterile centrifuge tube. Prepare

1×Anti-HSA:HRP freshly, gently mix the solution by inverting the tube and use immediately.

(3) Preparation of Calibration Standard solutions

- Prepare HSA Calibration Standard solutions as indicated in Fig 3 and Table 3.

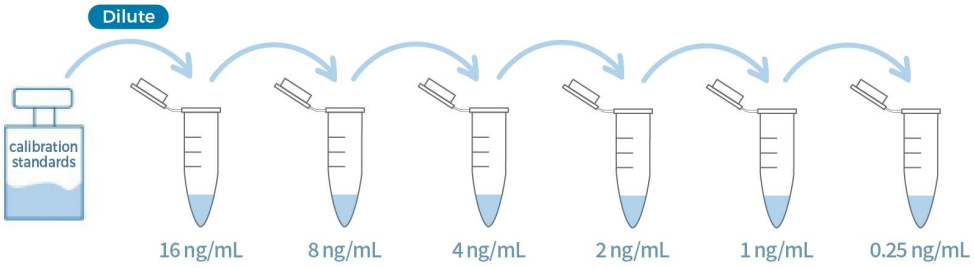


Figure 3. Graphic scheme of HSA Calibration Standard solutions

Table 3. Preparation of HSA Calibration Standard solutions

Tubes	Dilution procedure	Conc. (ng/mL)
ST1	Dilute the HSA Calibration Standard to ST1	16
ST2	500 µL ST1 + 500 µL Diluent	8
ST3	500 µL ST2 + 500 µL Diluent	4
ST4	500 µL ST3 + 500 µL Diluent	2
ST5	500 µL ST4 + 500 µL Diluent	1
ST6	200 µL ST5 + 600 µL Diluent	0.25
NCS	Diluent	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 samples stability studies to prevent degradation or denaturation during the experiment. Avoid repeated freeze-thaw cycles. Long-term storage at -70°C or below is recommended to avoid degradation.
- Dilute the samples with a suitable diluent to achieve a proper range of HSA 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 Loading

- Pipette 100 μL of 1×Anti-HSA:HRP Solution into each designated well according to the experiment design.
- Pipette 100 μL of Calibration Standard solutions, controls and samples into the corresponding wells as indicated earlier. Avoid foaming bubbles during pipetting. We recommend to prepare prepare 2-3 replicates for each concentration.
- Seal the plate and incubate on microplate thermoshaker at 500-600 rpm for 2 hours at room temperature, and protect from light.

Table 4. Example of 96-well plate 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 spiked test samples.
- ✧ The number of replicates and the involvement of spiked samples can be determined by conducting a method validation.

(2) Substrate Incubation

- Equilibrate the TMB substrate for 20 min at room temperature.
- Wash the plate with 1×Wash Buffer for about 340 μL each well. 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 wells, and incubate at room temperature for

10 minutes, and protect from light.

Note: Do not use sealing film during this step.

(3) Termination

- Add 50 μL of Stop Solution into each well.

  Note: The adding sequence should be the same as the adding sequence of the TMB solution. Suspend the tips while adding samples to prevent contact with the solution in the wells and minimize the risk of bubble formation.

(4) 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 duplicate 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 HSA 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.

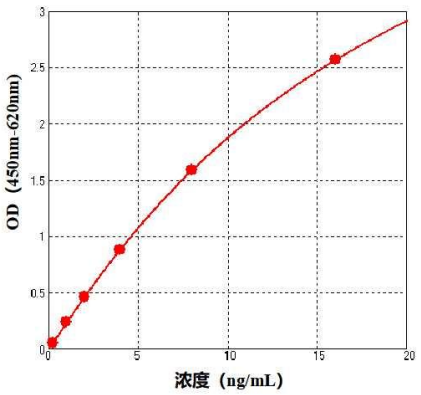
■ Limitations

- For research purposes only, but not intended for clinical use.
- Recommend sample pH between 6.0 and 8.0. Beyond this range may cause abnormal results.
- The kit is highly sensitive and specifically designed for detecting trace amounts of HSA in biological products, including natural and recombinant HSA. A method validation is required for other types of biological samples.

■ Assay Performance

- Linearity & Range: 0.25-16 ng/mL, $R^2 \geq 0.990$.
- LLOQ: 0.25 ng/mL.
- Specificity: No cross-reactivity with fetal bovine serum, fetal bovine serum and horse serum.
- Typical calibration curve and results:

Concentration（ng/mL）	$OD_{450\ nm-620\ nm}$	AVG
16	2.6858	2.5928
	2.5237	
	2.5688	
8	1.6479	1.6182
	1.5852	
	1.6215	
4	0.9538	0.9084
	0.8888	
	0.8826	
2	0.501	0.4890
	0.4804	
	0.4855	
1	0.2867	0.2668
	0.2631	
	0.2506	
0.25	0.0882	0.0849
	0.0839	
	0.0825	
0	0.0270	0.0256
	0.0271	
	0.0228	



方程: y = (A - D) / [1 + (x/C)^B] + D

A = 5.91069

B = -1.06512

C = 20.56331

D = 0.00881

R^2 = 0.99998

■ Additional Information

- ✧ This kit is intended for use by qualified technicians only.
- ✧ Use sterile disposable tips, tubes and reservoirs, etc. separately. 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℃.
- ✧ Seal or cover the microplate immediately after sample loading to avoid liquid evaporation.
- ✧ Avoid drying the wells before substrate incubation.
- ✧ Store unused microtiter strips in a sealed bag with desiccant to prevent contamination.
- ✧ Centrifuge Anti-HSA:HRP (100×) before use avoid any loss of the reagent.
- ✧ To avoid pipetting errors, pipette or sampling accurately for dilution of standards and samples, for example, minimum volume of 5 μL is recommended.
- ✧ HSA Calibration Standard solutions and anti-HSA Antibody solution are recommended for single use due to instability 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. Bubbles can influence optical density values and detection results.
- ✧ Reading should be completed within 30 minutes after termination.
- ✧ Avoid the samples containing sodium azide (NaN_3), which will deactivate the HRP and lead to the underestimation of HSA 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 micropipettes 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 more 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	Add the samples to the bottom of the wells using micropipettes, 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

Effective date: 08 Jul. 2024

Support & Contact

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