Vero HCP ELISA Kit

（One-step ELISA）

User Guide

PLEASE READ THE DOCUMENT CAREFULLY BEFORE EXPERIMENT

Product No.: 1301309
Version: A/1
For Research Use Only

Huzhou Shenke Biotechnology Co., Ltd.

■ Product Name

Vero HCP ELISA Kit (One-step ELISA)

■ Package

96 tests/Kit

■ Intended Use

This kit is suitable for the quantitation of Vero cell derived host cell proteins (HCPs) in various bioproducts, produced by sonication cell lysis.

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 host cell proteins (HCPs) from vero cells. A sheep polyclonal antibody specific to vero HCPs was employed in the assay to capture any remaining HCPs in the sample. The antibody coverage is assessed by the current mainstream method. Both the Calibration Standard (or test sample) and the HRP (Horseradish Peroxidase) labeled anti-Vero HCP antibody were simultaneously added to the microtiter plate, which coated with the affinity purified capture antibody and followed by incubation and washing. 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 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 450nm). The absorbance values at 450nm wavelength were positively correlated with the HCPs concentration in the Calibration Standard and the sample. The concentration of HCPs in the sample can be calculated using the dose-response curve.

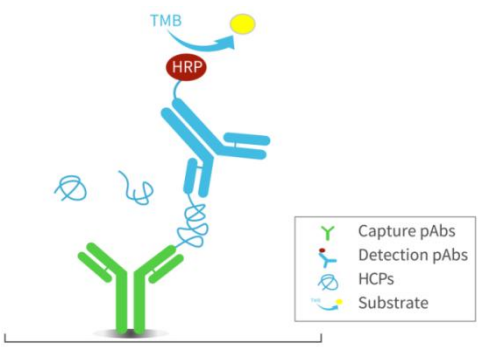


Figure 1. Schematic diagram

■ Kit Contents

Table 1.Kit Components

Reagent	Part No.	Quantity	Note
Vero HCP Calibration Standard	PNB012	2 bottles	Lyophilized powder. Dissolve it with the reconstitution solution (500 μL), and let it stand for about 5 minutes until transparent. Please refer to the details on the label of the tube.
Anti-Vero HCP Microtiter Strips	PNA013	8 well ×12 strips	Strips pre-coated with sheep anti-Vero HCP affinity antibody in a vacuumed bag with desiccant. Seal and store immediately after use.
Reconstitution Solution	PNC002	1×1.5 mL	Only used for dissolving Vero HCP Calibration Standard.
Diluent	PNE004	2×25 mL	For dilution of Calibration Standard, Anti-Vero:HRP(100×) and samples.
Wash Buffer Concentrate(10×)	PNF001	2×25 mL	Easy to be crystallized at low temperature, please incubate at 37℃ in water bath before use. Dilute 10 times with freshly prepared ultra-pure water to obtain 1×Wash Buffer Solution.
Anti-Vero :HRP (100×)	PNN007	1×120 μL	Affinity purified sheep antibody conjugated to HRP in a protein matrix with preservative. Dilute 100 times in diluent (PNE004) before use.
TMB Substrate	PND005	1×12 mL	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.
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-Vero HCP microtiter strips	Store in the bag with desiccant at 2-8℃ for up to 30 days.
Reconstituted Vero HCP Calibration Standard	Store at -20℃ , freeze and thaw for no more than 3 times.

■ Materials Required But Not Provided

- Sterile centrifuge tubes for dilution
- Absorbent paper for plate drying
- Pipette Tips: 1000 μL, 100 μL, 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
- Microplate thermoshaker
- Incubator (optional)
- Plate washer (optional)

■ Workflow

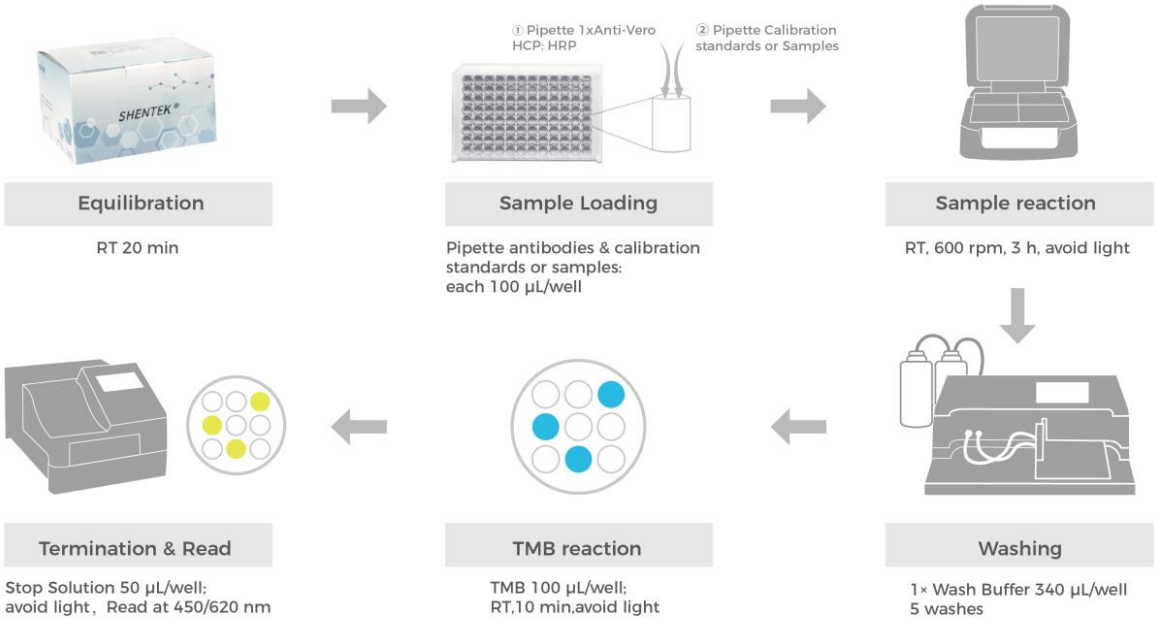


Figure 2. Procedure Flowchart

1. Preparation

(1) Equilibration

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

- Vero HCP Calibration Standard Solution: Pipette 500 µL of Reconstitution Solution into the bottle containing Vero HCP Calibration Standard. Gently invert 3-5 times to mix and let it stand for 5 minutes. Save the remaining solution under the recommended condition.

  Note: Do not use any other volumes of Reconstitution Solution to dissolve the Calibration Standard.
- 1× Wash Buffer: Dilute 1 volume of Wash Buffer Concentrate (10×) with 9 volumes of ultra-pure water. For example, add 25mL Wash Buffer Concentrate (10×) to 225mL of ultra-pure water to make 250mL 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℃ until it clear.

- 1×Anti-Vero :HRP: Prepare the 1×Anti-Vero :HRP by diluting the Anti-Vero :HRP (100×) with Diluent in a sterile centrifuge tube. Prepare 1×Anti-Vero :HRP fresh, mix gently and use it immediately.

(3) Preparation of Calibration Standard Solutions

- Prepare Vero HCP Calibration Standard Solutions as indicated in Fig 3 and Table 3.

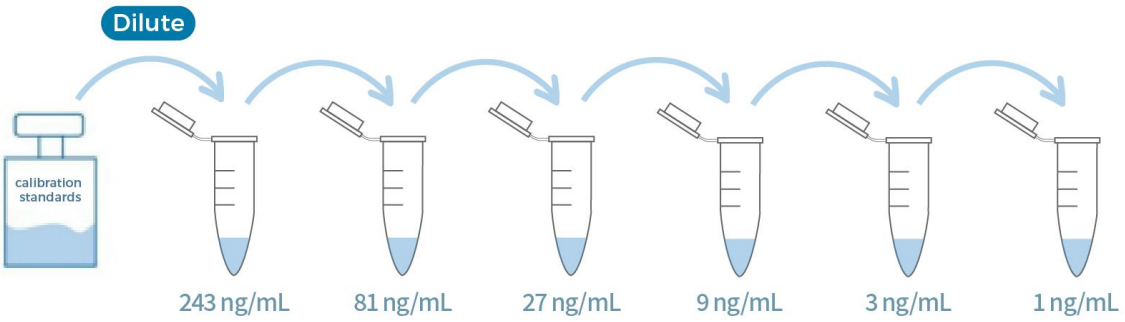


Figure 3.Graphic scheme of Vero HCP Calibration Standard Solutions

Table 3. Preparation of Vero HCP Calibration Standard Solutions

Tubes	Dilution procedure	Conc.(ng/mL)
ST1	Dilute reconstituted Vero HCP Calibration Standard to ST1	243
ST2	300 µL ST1 + 600 µL Diluent	81
ST3	300 µL ST2 + 600 µL Diluent	27
ST4	300 µL ST3 + 600 µL Diluent	9
ST5	300 µL ST4 + 600 µL Diluent	3
ST6	300 µL ST5 + 600 µL Diluent	1*
NCS	Diluent	0

*Anchor point

(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-thaw cycles. For long-term storage, -70°C is recommended to avoid degradation.
- Dilute the samples with a suitable diluent to achieve a proper range of HCP 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-Vero :HRP Solution into each designated well according to the experimental design.
- Pipette 100 μL of Calibration Standard solutions, controls and samples into the corresponding wells as indicated earlier. Avoid foaming bubbles during pipetting. It is recommended to prepare 2-3 parallels for each concentration.
- Seal the plate and incubate on microplate thermoshaker at 600 rpm for 3 hours at room temperature and protect from light.

Table4. Example of the layout of 96 tests kit.

	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” means the spiked recovery controls for each sample.

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

(2) Substrate Incubation

- Equilibrate the TMB substrate for 20 min at room temperature.
- Wash the plate with 340 μL of 1×Wash Buffer per 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 dry before adding the substrate.
- Add 100 μL of TMB Substrate into the wells, and incubate at RT for 10 minutes, 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 order of adding stop solution should be the same as the order of adding the TMB solution. 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.

(4) Reading

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

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 recommended to use professional standard curve software such as Curve Expert, ELISA Calc, and so on.

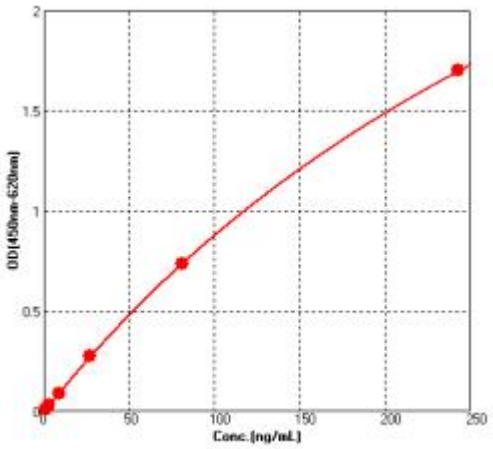
■ Limitations

- This product is intended for research use only but not for clinical use.
- The samples pH should be between 6.5 and 8.5. Beyond this range may cause abnormal results.

■ Assay Performance

- Linearity& Range: 3-243 ng/mL，$R^2 \geq 0.990$
- LLOQ: 3 ng/mL
- Specificity: No cross-reactivity with *E.coli, P.pastoris* and Sf9 cell.
- Typical calibration curve and data for reference:

Calibration Standards(ng/mL)	Abs. at 450nm-620nm	AVG
0	0.0561	0.0546
	0.0541	
	0.0536	
1	0.0647	0.0639
	0.0656	
	0.0613	
3	0.0866	0.0842
	0.0826	
	0.0834	
9	0.1432	0.1450
	0.1512	
	0.1405	
27	0.3283	0.3298
	0.3335	
	0.3277	
81	0.8157	0.7929
	0.7823	
	0.7806	
243	1.7484	1.7538
	1.7794	
	1.7335	



■ Additional Information

- ✧ This kit is intended for use by qualified technicians only.
- ✧ 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℃.
- ✧ 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-Vero :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.
- ✧ Vero HCP Calibration Standard Solutions and anti-Vero HCP 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. 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.
- ✧ 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 HCP levels.

■ Troubleshooting

Problem	Possible Cause	Solution
High background signal(OD)	Cross-contamination of reagents, including distilled 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 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
- USP<1132>Residual Host Cell Protein Measurement in Biopharmaceutical
- EP<2.6.34> HOST-CELL PROTEIN ASSAYS
- ChP<9012>Guidance of Quantitative Method Validation for Biological Samples

Effective date:10 Jul. 2024

Support & Contact

Huzhou Shenke Biotechnology Co., Ltd.
www.shentekbio.com
Address: 8th Floor, 6B Building, No.1366 Hongfeng Road, Huzhou313000, Zhejiang Province, China
E-mail: info@shentekbio.com
Phone: +1 (908) 822-3199 / (+86) 400-878-2189