HEK293 HCP ELISA Kit

（One-step ELISA）

User Guide

PLEASE READ THE DOCUMENT CAREFULLY BEFORE EXPERIMENT

Product No.: 1301311
Version: A/1
For Research Use Only

Huzhou Shenke Biotechnology Co., Ltd

■ Product Name

HEK293 HCP ELISA Kit (One-step ELISA)

■ Package

96 tests/Kit

■ Intended Use

This kit is intended for use in determining the presence of host cell proteins (HCPs) in products manufactured by expression in HEK293 derived host cells, including but not limited to recombinant proteins, cell and gene therapy products.

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

■ Product Description

This kit utilizes a solid-phase Enzyme-linked Immunosorbent Assay (ELISA) with a double-antibody sandwich technique to detect residual host cell proteins (HCPs) from HEK293 cells in the sample. A polyclonal antibody specific to HEK293 HCPs was employed in the assay to capture any remaining HCPs in the sample. Both the Calibration Standard (or test sample) and the HRP (Horseradish Peroxidase) labeled anti-HEK293 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.

No special treatment is required for the test sample, and its suitability could be verified by the appropriate dilution ratios with the kit.

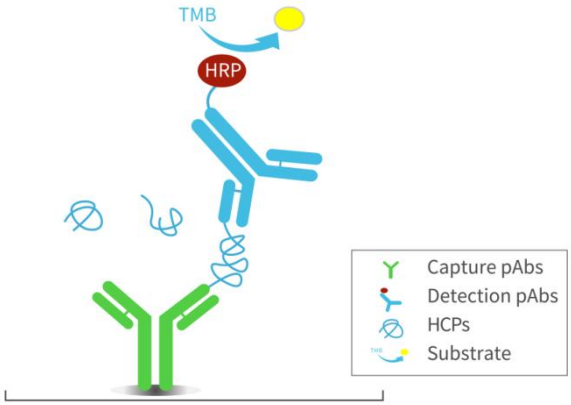


Figure 1. Schematic diagram

■ Kit Contents

Table 1. Kit Components

Reagent	Part No.	Quantity	Note
HEK293 HCP Calibration Standard	PNB008	3 bottles	Lyophilized powder. Dissolve it with 500 µL Reconstitution Solution and let it stand for about 5 minutes. The solution should be clear and transparent. Please refer to the bottle label for details.
Anti-HEK293 HCP Microtiter Strips	PNA011	8 well ×12 strips	Strips pre-coated with sheep anti-HEK293 HCP affinity antibody in a vacuumed bag with desiccant. Seal and store immediately after use.
Reconstitution Solution	PNC002	2×1.5 mL	Only used to dissolve HEK293 HCP Calibration Standard.
Diluent	PNE004	2×25 mL	For dilution of Calibration Standard, Anti-HEK293:HRP (100×) and samples.
Wash Buffer Concentrate (10×)	PNF001	1×25 mL	Easy to be crystallized at low temperatures, and can be dissolved in 37℃ water bath before use. Dilute at 1:10 with freshly prepared ultra-pure water for plate washing.

Anti-HEK293:HRP (100×)	PNN005	1×120 μL	Affinity purified sheep antibody conjugated to HRP. Dilute 100 times before use.
TMB Substrate	PND004	1×12 mL	Equilibrate to room temperature for 20 minutes before use. Keep away from light and sealed.
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℃. Use within the expiration date labeled upon the kit package. The opened components should be stored as follows.

Table 2. Recommended storage conditions for opened components

Component	Stability
Anti-HEK293 HCP Microtiter Strips	Store in the vacuumed bag with desiccant at 2-8℃ for up to 60 days.
Reconstituted HEK293 HCP Calibration Standard	Store at -20℃ and no more than 3 freeze-thaw cycles

■ Materials Required But Not Supplied in the Kit

- Sterile centrifuge tubes for dilution
- Absorbent paper for plate drying
- Sterile pipette tips
- 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

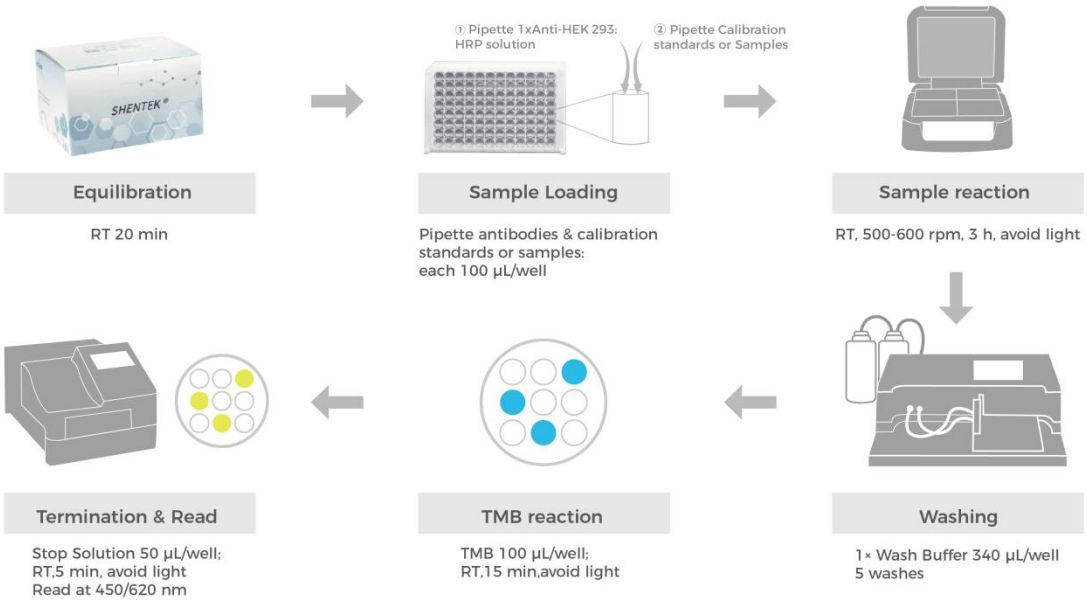


Figure 2. Procedure Flowchart

1. Preparation

(1) Equilibration

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

(2) Preparation of Reagents

- HEK293 HCP Calibration Standard solution: Pipette 500 µL of Reconstitution Solution into the bottle containing HEK293 HCP Calibration Standard. Gently invert to mix and let it stand for 5 minutes. Save the remaining solution under the recommended condition.

  Note: Do not use other volumes of Reconstitution Solution to dissolve the

Calibration Standard.

- 1× Wash Buffer: Dilute the Wash Buffer Concentrate (10×) at 1:10 with ultra-pure water. For example, add 25mL 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. It can be purchased separately.

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

- 1×Anti-HEK293:HRP: Prepare the 1×HEK293:HRP by diluting the Anti-HEK293:HRP (100×) with Diluent in a sterile centrifuge tube. Gently mix the solution and use it immediately.

  Note: Anti-HEK293:HRP (100×) need to equilibrate at room temperature for 20 minutes, and return to 2-8℃ after use.

(3) Preparation of Calibration Standard Solutions

- According to Fig 3 and Table 3, prepare HEK293 HCP Calibration Standard Solutions.

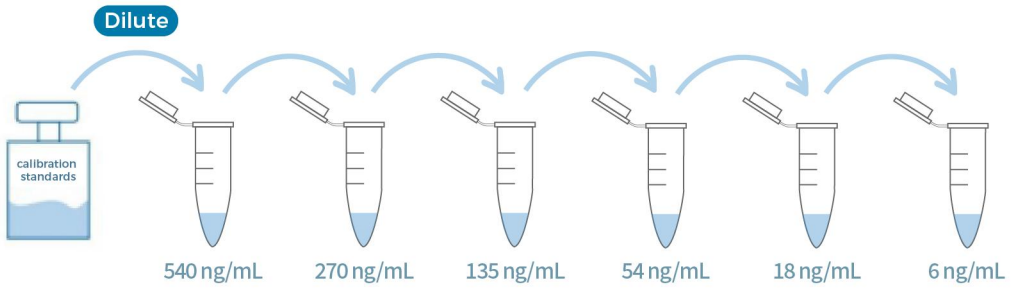


Figure 3. Graphic scheme of HEK 293 HCP Calibration Standard Solutions

Table 3. Preparation of HEK 293 HCP Calibration Standard Solutions

Serial Dilution Tube	Dilution procedure	Conc. (ng/mL)
ST1	Dilute the reconstituted HEK293 HCP Calibration Standard to ST1	540
ST2	500 µL ST1 + 500 µL Diluent	270
ST3	500 µL ST2 + 500 µL Diluent	135
ST4	360 µL ST3 + 540 µL Diluent	54
ST5	300 µL ST4 + 600µL Diluent	18
ST6	300 µL ST5 + 600 µL Diluent	6
NCS	Diluent	0

(4) Sample Preparation

- Test samples: In-process samples, drug substance and drug product. Samples should be clear and transparent, and insoluble substances need to be removed from samples through centrifugation or filtration.
- Conduct sample stability studies to prevent degradation or denaturation during the experiment. Long-term storage at -70°C is recommended to avoid degradation, and avoid repeated freeze-thaw cycles.
- Dilute the samples with a suitable diluent to achieve a concentration of host cell proteins (HCPs) within the quantification range of the calibration curve.
- Recommend to verify sample suitability by determining the appropriate sample dilution factor before the initial test and facilitating the subsequent routine testing.

Note: Please contact us for support of validation protocol.

2. Assay Experiment

(1) Sample Loading

- Pipette 100 µL of 1×Anti-HEK293:HRP Solution into each designated well according to the experimental design.
- Pipette 100 µL of Calibration Standard, 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 500-600 rpm for 3 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" means 6 concentration gradients, "NCS" means negative control, "S1-S3" means test samples , and "S1 SRC-S3 SRC" means the spiked test samples.
- ✧ The number of replicates and the inclusion of spiked samples can be determined based on the results of method validation.

(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. Repeat washing for 5 times. Wipe off any liquid from the bottom outside of the plate. Do not allow the wells to dry before adding the substrate.
- Add 100 μL of TMB Substrate into wells, and incubate at room temperature for 15 minutes, protect from light.

  Note : Do not use sealing film for 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.
- Incubate at room temperature for another 5 minutes, protect from light.

(4) Reading

- Read absorbance at 450/620-650 nm.

3. Calculation and Analysis

- The OD_{450nm} values of each well should be subtracted by their respective long wavelength OD values. If the microplate reader is not equipped with long wavelength measurement, this step can be omitted.
- After subtracting the OD value of the negative control from the OD values of each calibration point and sample, calculate 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 analysis of the standard curve could be the one that comes with the microplate reader. If not, it is recommended to use professional standard curve software such as Curve Expert, ELISA Calc, and so on.
- For samples with absorbance values exceeding the Calibration Standard ST1, the appropriate dilution should be performed before retesting. The HCP 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.

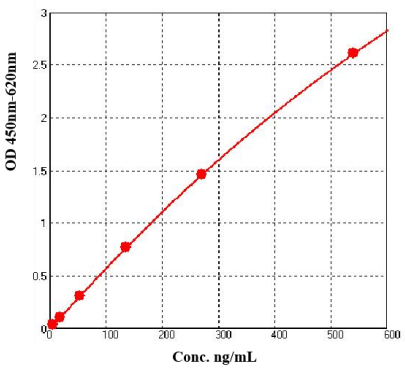
■ Limitations

- For research purposes only; not intended for clinical use.
- Specifically designed for detecting residual protein content in products manufactured from HEK293 host cells. A method validation is required before relying exclusively on this assay.
- Recommend sample pH between 6.5 and 8.5, and measurements may be compromised if the sample pH is too low or too high.

■ Assay Performance

- Linearity & Range: 6-540 ng/mL, R^2>0.990.
- LLOQ: 6 ng/mL.
- Typical calibration curve and results:

Calibration Standards (ng/mL)	Abs. At (450 nm-620 nm)	AVG
540	2.5612	2.6488
	2.7048	
	2.6805	
270	1.4940	1.4994
	1.5070	
	1.4972	
135	0.8101	0.8078
	0.8022	
	0.8110	
54	0.3522	0.3492
	0.3562	
	0.3392	
18	0.1507	0.1476
	0.1477	
	0.1445	
6	0.0769	0.0756
	0.0749	
	0.0749	
0	0.0417	0.0405
	0.0401	
	0.0396	



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

A = 10.12035
B = -1.04527
C = 1488.28609
D = 0.00489
R^2 = 1.00000

■ 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 notmix 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-HEK 293:HRP(100×) before use avoid any loss of the reagent.
- ✧ Accurately pipetting or sampling for dilution of standards and samples, for example, minimum volume of 5 μL is recommended.
- ✧ HEK293 HCP Calibration Standard and 1×Anti-HEK293:HRP 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 assitance.
- ✧ Pipette carefully to avoid any bubbles, and gently shake the plate for thorough mixing. Sometimes air, resulting in bubbles, can be drawn into the pipette or dispensed into the wells. If this happens, bubbles can influence optical density values and 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 equipments, 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 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 any other difficulties, 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 Biopharmaceuticals
- EP<2.6.34> HOST-CELL PROTEIN ASSAYS
- ChP<9012> Guidance of Quantitative Method Validation for Biological Samples

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Support & Contact

SHENTEK

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