SHENTEK

CHO-K1 HCP ELISA Kit
(One-step ELISA)
User Guide

PLEASE READ THE DOCUMENT CAREFULLY BEFORE EXPERIMENT

Product No.: 1301305
Version: A/1
For Research Use Only

Huzhou Shenke Biotechnology Co., Ltd

Product Name

CHO-K1 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 using the Chinese Hamster Ovary-K1 (CHO-K1) cell line.
The kit is for RESEARCH USE ONLY and not intended for clinical use.

Product Description

The kit employs a solid-phase Enzyme-linked Immunosorbent Assay (ELISA) with a double-antibody sandwich technique to detect residual CHO-K1 host cell proteins in the samples. Polyclonal antibody specific to CHO-K1 HCPs was employed in the assay to capture any remaining HCPs in the samples. Both the Calibration Standard (or test samples) and the HRP (Horseradish Peroxidase) labeled anti-CHO-K1 HCPs 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 the reaction, HRP catalyzed the oxidation of TMB by H_2O_2 to produce a blue product (maximum absorption peak at 655 nm). The stop solution is added to terminate the enzymatic reaction, resulting in a yellow color product (maximum absorption peak at 450 nm). The absorbance values at 450 nm wavelength were positively correlated with the HCPs concentration in the Calibration Standard and the samples. The concentration of CHO-K1 HCPs in the samples can be calculated using the dose-response curve.
No special treatment is required for the test samples and its suitability could be verified by appropriate dilutions with the kit.

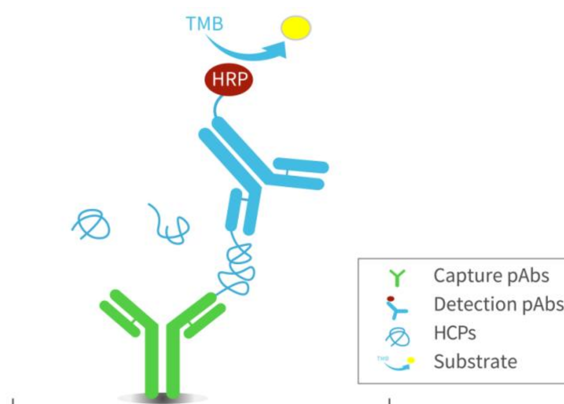


Figure 1. Schematic diagram

■ Kit Contents

Table 1. Kit Components

No.	Reagent	Part No.	Quantity	Note
I	CHO-K1 HCP Calibration Standard	PNB017	1 × 50 μL	0.5 mg/mL. Please refer to the details on the label of the tube.
	Anti-CHO-K1:HRP (250×)	PNN011	1 × 50 μL	Purified anti-CHO-K1 HCP goat antibody conjugated to HRP. Dilute 250 times in Diluent before use.
II	Anti-CHO-K1 HCP Microtiter Strips	PNA021	8 well × 12 strips	Strips pre-coated with goat anti-CHO-K1 HCP antibody in a vacuumed bag with desiccant. Seal and store immediately after use.
	Wash Buffer Concentrate (20×)	PNF002	1 × 15 mL	Dilute 20 times in freshly prepared ultra-pure water for plate washing.
	TMB Substrate	PND006	1 × 12 mL	Equilibrate to room temperature for 20 minutes before use. Sealed and keep away from light.
	BSA	PNQ001	1 × 1 g	Used to prepare Diluent.
	Stop Solution	PNI003	1 × 12 mL	Sulfuric acid solution. Avoid direct contact with eyes, skin, and clothing.
	Sealing Film	PNK002	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 I at -20℃ and kit II at 2-8℃. Please check the expiration date on the labels.
- The validity period of prepared Diluent is 7 days.
- CHO-K1 HCP Calibration Standard and Anti-CHO-K1:HRP (250×) are freezed-thaw for not 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 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
- Microplate thermoshaker
- Incubator (optional)
- Plate washer (optional)
- Water bath (optional)

■ Workflow

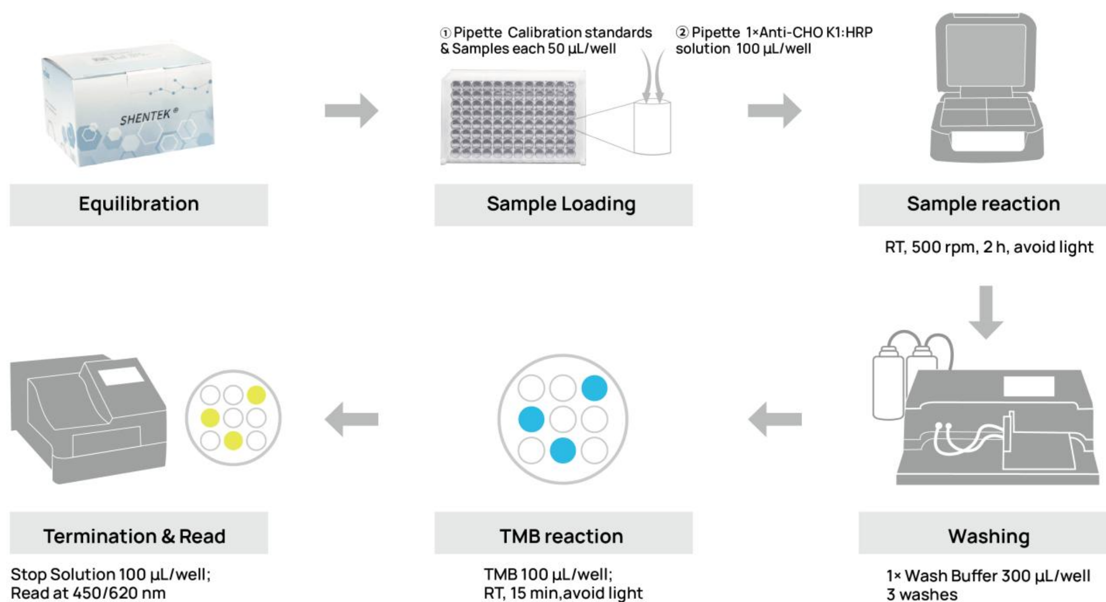


Figure 2. Procedure Flowchart

1. Preparation

(1) Equilibration

- Before use, allow the kit to equilibrate at room temperature for 20 minutes. Ensure that the Calibration Standard and Anti-CHO-K1:HRP (250×) are completely thawed before use, and return to appropriate storage temperature after use.
- Take the appropriate amount of strips to a strip holder according to the experimental design and 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 Concentrate (20×) with 19 volumes of ultra-pure water. For example, add 15 mL Wash Buffer Concentrate (20×) to 285 mL of ultra-pure water to prepare 300 mL of 1×Wash Buffer. Mix well before use.
- Diluent: Dissolve 1 g of BSA in 100 mL of 1×Wash Buffer. Mix well before use and store at 2-8°C. The prepared Diluent is valid for 7 days, and it is recommended to prepare it as needed.

Note: If the Wash Buffer Concentrate (20×) or Diluent is cloudy or contains precipitates, heat at 37°C until it clears.

- 1×Anti-CHO-K1:HRP: Prepare the 1×Anti-CHO-K1:HRP by diluting the Anti-CHO-K1:HRP (250×) with Diluent in a sterile centrifuge tube. Gently mix the solution and use it immediately.

(3) Preparation of Calibration Standard Solutions

- Prepare CHO-K1 HCP Calibration Standard Solutions according to Figure 3 and Table 3.

Figure 3. Graphic scheme of CHO-K1 HCP Calibration Standard Solutions

Table 3. Preparation of CHO-K1 HCP Calibration Standard Solutions

Serial Dilution Tube	Dilution procedure	Conc. (ng/mL)
A	10 μL Calibration Standard + 490 μL Diluent	10000
ST1	10 μL A + 990 μL Diluent	100
ST2	300 μL ST1 + 100 μL Diluent	75
ST3	200 μL ST2 + 175 μL Diluent	40
ST4	150 μL ST3 + 350 μL Diluent	12
ST5	200 μL ST4 + 200 μL Diluent	6
ST6	200 μL ST5 + 200 μL Diluent	3
NCS	Diluent	0

(4) Sample Preparation

- Test samples: Cell culture harvested bulk, in-process samples, drug substance and drug product. Samples should be 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 or below 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 50 µL of Calibration Standard Solutions 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.
- Pipette 100 µL of 1×Anti-CHO-K1:HRP into each designated well according to the experiment design.
- Seal the plate and incubate on microplate thermoshaker at 500 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									
B												
C	ST6	ST6	ST6	S1	S1	S1						
D	ST5	ST5	ST5	S2	S2	S2						
E	ST4	ST4	ST4	S3	S3	S3						
F	ST3	ST3	ST3	S1+SRC	S1+SRC	S1+SRC						
G	ST2	ST2	ST2	S2+SRC	S2+SRC	S2+SRC						
H	ST1	ST1	ST1	S3+SRC	S3+SRC	S3+SRC						

- ✧ "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 spiked samples can be determined by method validation.

(2) Substrate Incubation

- Equilibrate the TMB Substrate for 20 minutes at room temperature.

- Wash the plate with 1×Wash Buffer for about 300 μL each well. Wipe off any liquid from the bottom outside of the plate. Repeat washing for 3 times. 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, and protect from light.

  Note: Do not use sealing film for this step.

(3) Termination

- Add 100 μL of Stop Solution into each well.

  Note: The adding sequence should be the same as the adding sequence of the TMB Substrate. While adding samples, suspend the tips above the liqid 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 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, we recommend to use professional standard curve software such as Curve Expert, ELISA Calc, and so on.
- 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

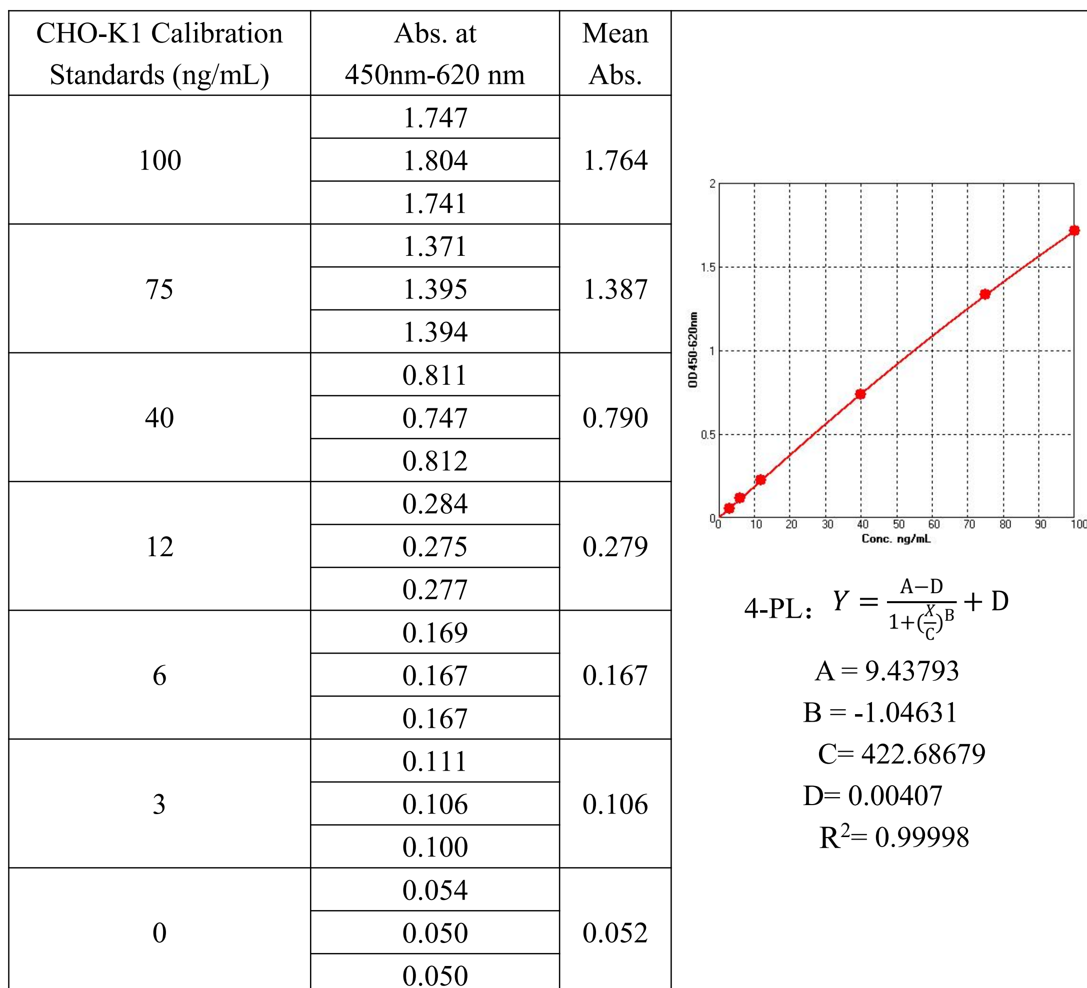
at this dilution level and the recovery rate should meet the requirements of the corresponding regulations.

Limitations

- For research purposes only but not intended for clinical use.
- Specifically designed for detecting residual protein from CHO-K1 cell production process.
- 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: 3 - 100 ng/mL, $R^2 \geq 0.990$.
- LLOQ: 3 ng/mL.
- Specificity: No cross-reactivity with host proteins from MDCK, Vero, HEK293T, *E.coli, P.pastoris* and Sf9.
- Typical calibration curve and results:



CHO-K1 Calibration Standards (ng/mL)	Abs. at 450nm-620 nm	Mean Abs.
100	1.747	1.764
	1.804	
	1.741	
75	1.371	1.387
	1.395	
	1.394	
40	0.811	0.790
	0.747	
	0.812	
12	0.284	0.279
	0.275	
	0.277	
6	0.169	0.167
	0.167	
	0.167	
3	0.111	0.106
	0.106	
	0.100	
0	0.054	0.052
	0.050	
	0.050	

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

A = 9.43793

B = -1.04631

C= 422.68679

D= 0.00407

R^2= 0.99998

■ 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 Sulfuric acid. 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 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-CHO-K1:HRP (250×) before use 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.
- ✧ 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 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 ultra-pure water	Freshly prepared prior to experiment.
	Cross-contamination of equipment,	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	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
- 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

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