CHO-K1 HCP ELISA Kit

(One-step ELISA)

User Guide

PLEASE READ THE DOCUMENT CAREFULLY BEFORE EXPERIMENT

Product No.:1301305-1
Version: A/0
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 protein (HCP) contamination in products derived from the CHO-K1 cell line.

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

■ Product Description

This kit employs a solid-phase enzyme-linked immunosorbent assay (ELISA) utilizing a double-antibody sandwich technique to quantify residual host cell proteins (HCPs) originated from the CHO-K1 cell line.

A sheep polyclonal antibody specific to CHO-K1 HCPs was employed in the assay to capture any remaining HCPs in the samples. Calibration standards or test samples, along with the HRP-labeled anti-CHO-K1 HCP antibody, were simultaneously added to the microtiter plate pre-coated with the affinity purified capture antibody, and followed by incubation and washing. TMB (3,3',5,5'-tetramethylbenzidine) substrate was added into the reaction, catalyzed by the HRP oxidation to produce a blue-colored 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 was positively correlated with the HCPs concentration in the calibration standard and the samples. The concentration of HCPs in the samples can be calculated using a dose-response curve.

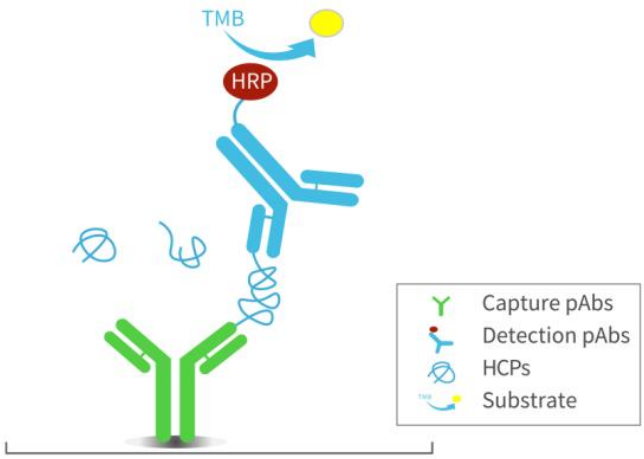


Figure 1. Schematic diagram

■ Kit Contents

Table 1. Kit Components

Reagent	Part No.	Quantity	Note
Anti-CHO-K1 HCP Microtiter Strips	PNA024	8 well × 12 strips	Strips pre-coated with sheep anti-CHO-K1 HCP antibody in a vacuumed bag with desiccant. Seal and store immediately after use.
CHO-K1 HCP Calibration Standard	PNB025	2 bottles	Lyophilized powder. Dissolve it with 500 μL Reconstitution Solution, and let it stand for about 5-10 minutes until transparent. Please refer to the details on the label of the tube.
Reconstitution Solution	PNC002	1 × 1.5 mL	Only used for dissolving CHO-K1 HCP Calibration Standard.
Diluent	PNE004	2 × 25 mL	For dilution of Calibration Standard, Anti-CHO-K1 HCP:HRP (100×) and samples.
Wash Buffer Concentrate (10×)	PNF001	2 × 25 mL	Dilute 10 times with freshly prepared ultra-pure water for plate washing.
Anti-CHO-K1 HCP:HRP (100×)	PNN014	1 × 120 μL	Affinity purified sheep antibody conjugated to HRP in a protein matrix with preservative. Dilute 100 times in Diluent before use.
TMB Substrate	PND004	1 × 12 mL	Seal and protect from light. Equilibrate to room temperature (RT) for 20 minutes before use.
Stop Solution	PNI003	1 × 12 mL	Avoid direct contact with eyes, skin, and clothing.
Sealing Film	PNK001	3 pieces	Cover the strips 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-CHO-K1 HCP Microtiter Strips	Store in the bag with desiccant at 2-8℃ for up to 60 days.
Reconstituted CHO-K1 HCP Calibration Standard	Aliquot the component and store it below -20℃ for up to 60 days. Avoid repeated freezing-thawing cycles.

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

■ Workflow

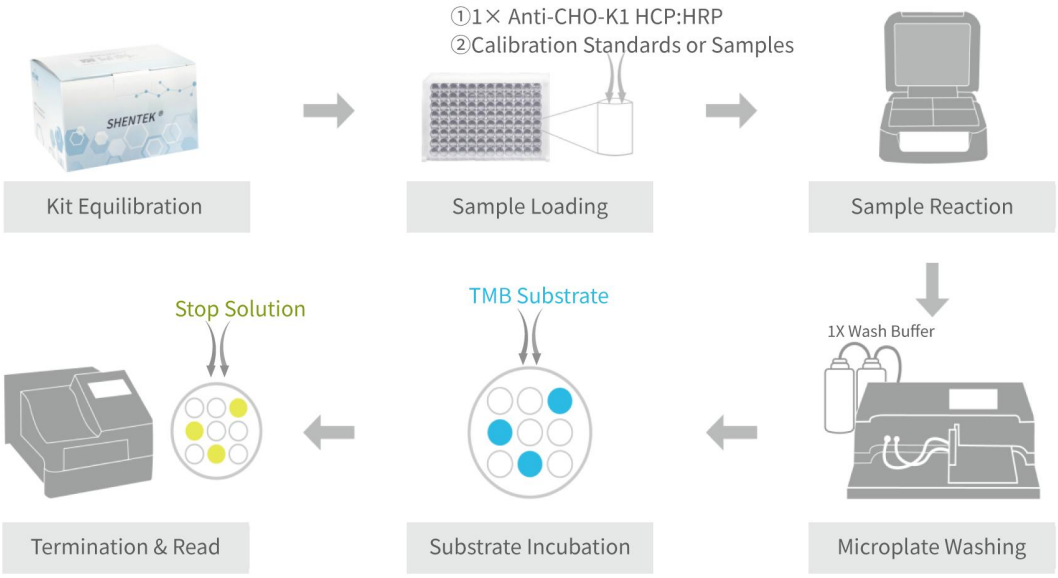


Figure 2. Procedure Flowchart

1. Preparation

(1) Equilibration

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

(2) Preparation of Reagents

- CHO-K1 HCP Calibration Standard Solution: Pipette 500 µL of Reconstitution Solution into the bottle containing CHO-K1 HCP Calibration Standard. Gently invert 3-5 times to mix well and let it stand for 5-10 minutes. Save the remaining solution under the recommended condition.

  Note:

  If two or more vials of calibration standards are applied, combined all after reco nstituted, and mix gently before use.

  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 25 mL Wash Buffer Concentrate (10×) to 225 mL of ultra-pure water to prepare 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-CHO-K1 HCP:HRP: Dilute the Anti-CHO-K1 HCP:HRP (100×) with Diluent in a sterile centrifuge tube to prepare the 1×Anti-CHO-K1 HCP:HRP, mix gently and prepare it freshly.

(3) Preparation of Calibration Standard Solutions

- Prepare CHO-K1 HCP Calibration Standard Solutions as shown in Fig 3 and Table 3.

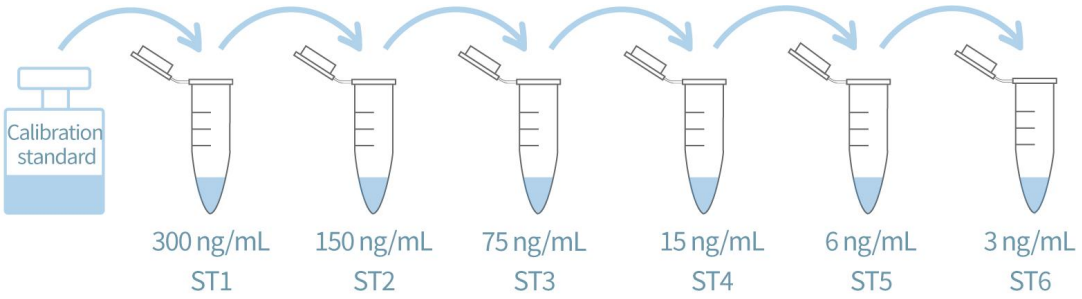


Figure 3. Graphic scheme of CHO-K1 HCP Calibration Standard Solutions

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

Tubes	Dilution Procedure	Conc. (ng/mL)
ST1	Dilute the reconstituted CHO-K1 HCP Calibration Standard to ST1 with Diluent	300
ST2	400 µL ST1 + 400 µL Diluent	150
ST3	400 µL ST2 + 400 µL Diluent	75
ST4	200 µL ST3 + 800 µL Diluent	15
ST5	300 µL ST4 + 450 µL Diluent	6
ST6	400 µL ST5 + 400 µL Diluent	3
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 sample stability studies to prevent degradation or denaturation during the experiment. Avoid repeated freeze-thraw 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 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 & Incubation

- Pipette 100 μL of 1×Anti-CHO-K1 HCP:HRP into each designated well according to the experimental design.
- Pipette 100 μL of Calibration Standard Solutions, NCS (Diluent) and samples into the corresponding wells as prepared earlier. Avoid foaming bubbles during pipetting. We recommend to prepare 2-3 replicates for each sample.
- Seal the plate and incubate on microplate thermoshaker at 600 rpm for 3 hours at room temperature and protect from light.

Table 4. Example of microplate 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” indicate 6 concentration gradients, “NCS” as negative control, “S1-S3” as test samples, and “S1+SRC-S3+SRC” as spiked recovery controls for each sample.

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

(2) Plate Washing & Color developing

- Equilibrate the TMB Substrate for 20 minutes at room temperature.
- Wash the plate with 300 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 dried before adding the substrate.
- Add 100 μL of TMB Substrate into the wells, and incubate at RT for 15 minutes, and protect from light.

  Note: Do not use sealing film during this step.

(3) Termination & Reading

- Add 50 μL of Stop Solution into each well, wait for 5 minutes and protect from light.
- 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 $OD_{450\ nm}$ 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.

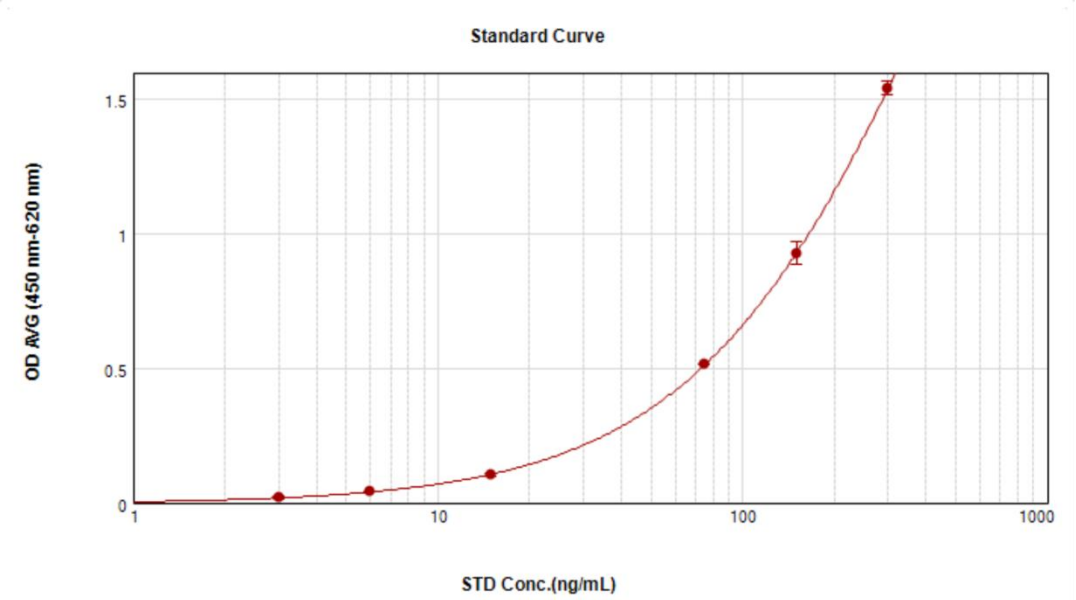
■ Limitations

- This product is intended for research use only but not for clinical applications.

- Specifically designed for detecting residual protein from CHO-K1 cell production process.
- The samples pH should be between 6.5 and 8.5. Beyond this range may cause abnormal results.

■ Assay Performance

- Linearity & Range: 3 - 300 ng/mL, $R^2 \geq 0.990$
- LLOQ: 3 ng/mL
- Specificity: No cross-reactivity with Sf9、HEK 293T、*E.coli* BL21、*P.pastoris* HCP.
- Typical calibration curve and results for reference:



Curve Fit Results ▲

Curve Fit : 4-Parameter Logistic $y = D + \frac{A-D}{1+\left(\frac{x}{C}\right)^B}$

	Parameter	Estimated Value	Std. Error	Confidence Interval
Std R^2 = 1.000 EC50 = 517.3	A	-0.003	0.005	[-0.024, 0.017]
	B	1.024	0.032	[0.885, 1.163]
	C	517.3	74.72	[195.8, 838.7]
	D	4.242	0.362	[2.684, 5.800]

■ Additional Information

- ✧ This kit is intended for lab 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℃.
- ✧ 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 HCP: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.
- ✧ CHO-K1 HCP Calibration Standard Solutions and 1×Anti-CHO-K1 HCP: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 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 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, including micropipette 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

- EP<2.6.34> HOST-CELL PROTEIN ASSAYS
- FDA. Bioanalytical Method Validation
- ICH. M10 Bioanalytical Method Validation And Study Sample Analysis
- JP<G3-9-172> Host Cell Protein Assay
- USP<1132> Residual Host Cell Protein Measurement in Biopharmaceuticals
- USP<1103> Immumological Test Methods Enzyme-Linked Immunosorbent Assay (Elisa)
- ChP. 9012. Guidance of Quantitative Method Validation for Biological Samples

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Support & Contact

Huzhou Shenke Biotechnology Co., Ltd.
www.shentekbio.com
Address: 8th Floor, 6B Building, No.1366 Hongfeng Road, Huzhou 313000, Zhejiang Province, China
E-mail: info@shentekbio.com
Phone: +1 (908) 822-3199 / (+86) 400-878-2189