CHO K1 HCP ELISA Kit

PLEASE READ THE DOCUMENT CAREFULLY BEFORE EXPERIMENT

Product No.: 1301305
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 (HCPs) in products manufactured with Chinese hamster ovary (CHO) cells.

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 [illegible] 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 [illegible] by incubation. [illegible] 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 standards 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.

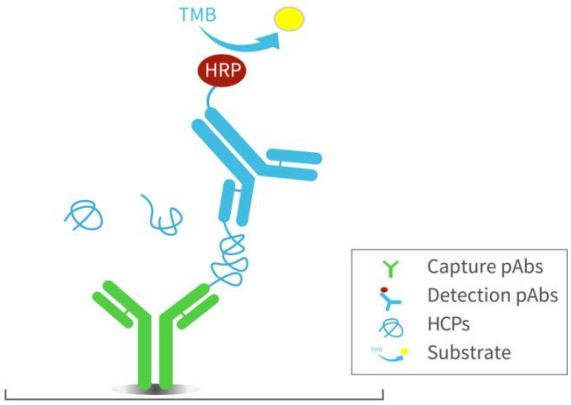


Figure 1. Schematic diagram

■ Kit Contents

Table 1. Kit Components

No.	Reagent	Part No.	Quantity	Note
	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 antibody conjugated to HRP. Dilute 250 times in Diluent before use.
	Anti-CHO K1 HCP Microtiter Strips	PNH021	8 well × 12 strips	Ready to use. Store with desiccant. Seal and store immediately after use.
II	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 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 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
- ➢ Incubator (optional)
- ➢ Plate washer (optional)
- ➢ Water bath (optional)

■ Workflow

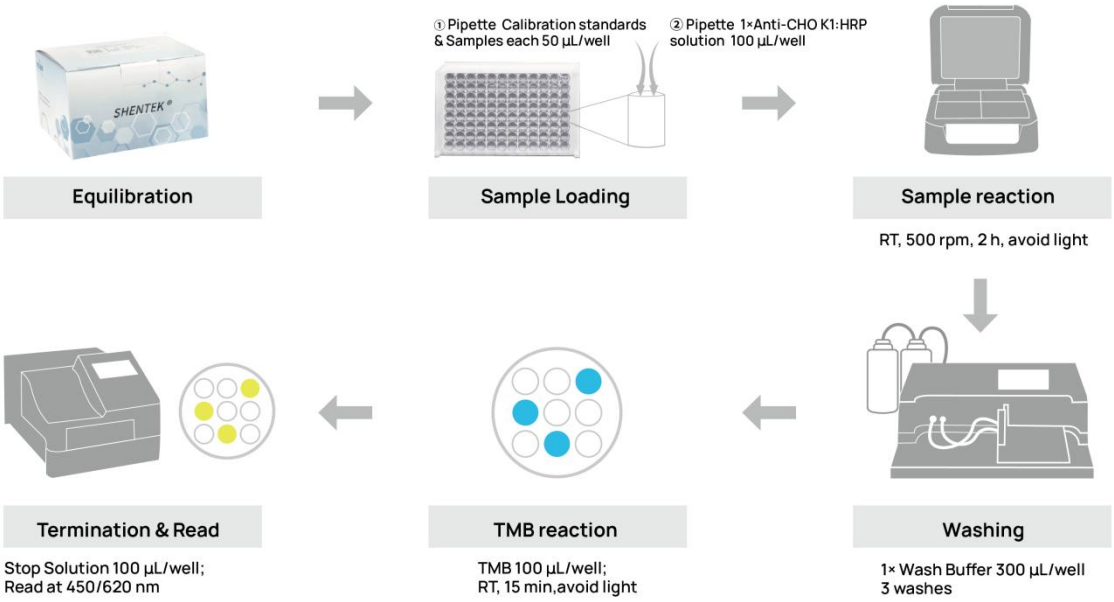


Figure 2. Procedure Flowchart

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℃.

(2) Preparation of Reagents

- 1×Wash Buffer: Dilute 1 volume of Wash Buffer 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℃. 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 standards Solutions

- Prepare CHO K1 HCP Calibration standard solutions according to Figure 3 and Table 3.

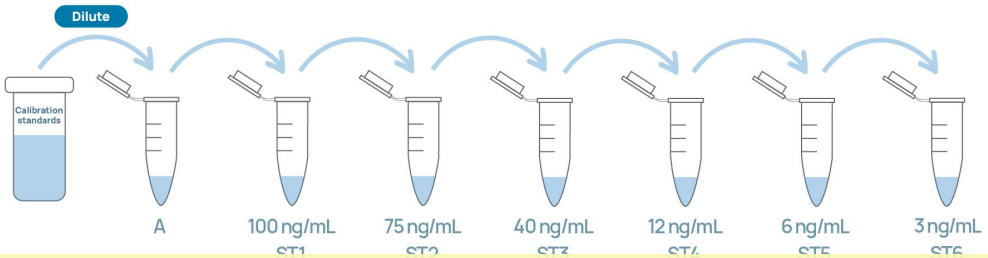


Figure 3. Graphic scheme of CHO K1 HCP Calibration standard solutions

Serial Dilution Tube	Dilution procedure	Conc. (ng/mL)
A	10 µL Calibration standard + 490 µL Diluent	10000
ST1	100 µL A + 100 µL Diluent	5000
ST2	200 µL ST1 + 200 µL Diluent	2500
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, 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.
- Pipette 100 µL of 1×Anti-CHO K1:HRP Solution 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									
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 min 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 solution. 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 measure [illegible]

- Subtract the OD value of the NCS from [illegible] record the mean of the replicate wells.

- Perform a 4-parameter logistic regression model using the Calibration standards 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.

- For samples with absorbance values exceeding the Calibration standard ST1, a pilot study 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.

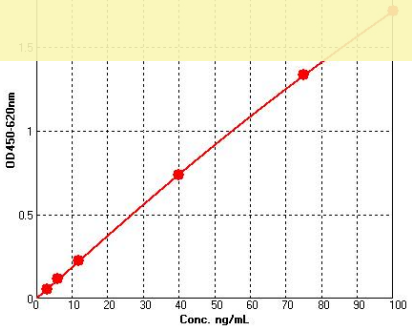
■ Limitations

- For research purposes only 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 MDCK, Vero, HEK293T, *E.coli*, *P.pastoris* and Sf9 cells.
- Typical calibration curve and results:

75	1.395	1.387
	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℃.
- ✧ 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 Kit-HRP (250×) before use to avoid product loss of the vessel.
- ✧ To avoid pipetting errors, pipette or sampling accurately for dilution of standards and samples. The minimum volume of 5 μl is recommended.
- ✧ Ensure there are no air bubbles in the wells. If not, remove them with a needle.
- ✧ Pipette carefully to avoid air bubbles, and remove any bubbles that may be present. 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 water	Freshly prepared prior to experiment.
	Cross-contamination of equipments, including mircopipettes 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.
	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 well and avoid splashing.
	Plate 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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