CHO HCP ELISA Kit

（One-step ELISA）

User Guide

PLEASE READ THE DOCUMENT CAREFULLY BEFORE EXPERIMENT

Product No.: 1301304-1
Version: A/1
For Research Use Only

Huzhou Shenke Biotechnology Co., Ltd

■ Product Name

CHO 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 CHO host cells, such as monoclonal antibodies, recombinant proteins, vaccines and so on.

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

■ Product Description

This kit utilizes a Fed-batch CHO (K1&S) cell culture to produce HCPs, which are used for the generation of specific antibodies via sheep immunization. The kit employs a solid-phase Enzyme-linked Immunosorbent Assay (ELISA) with a double-antibody sandwich technique to detect residual CHO host cell proteins (HCPs) in the sample. Polyclonal antibody specific to CHO 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-CHO 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 added for 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 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 sample. The concentration of CHO 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.

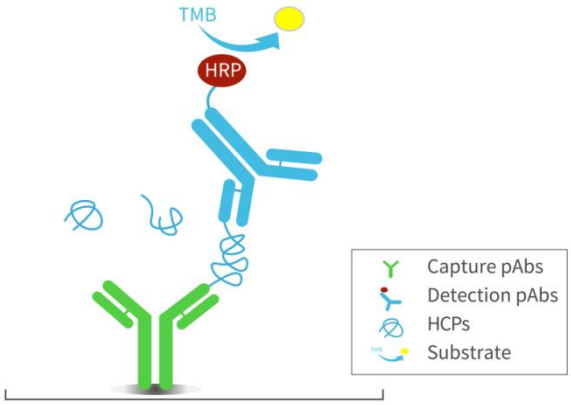


Figure 1. Schematic diagram

■ Kit Contents

Table 1. Kit Components

Reagent	Part No.	Quantity	Note
CHO HCP Calibration Standard	PNB005	2 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-CHO HCP Microtiter Strips	PNA008	8 well ×12 strips	Strips pre-coated with sheep anti-CHO HCP affinity antibody in a vacuumed bag with desiccant. Seal and store immediately after use.
Reconstitution Solution	PNC002	1×1.5 mL	Only used to dissolve CHO HCP Calibration Standard.
Diluent	PNE004	2×25 mL	For dilution of Calibration Standard, Anti-CHO:HRP (100×) and samples.
Wash Buffer Concentrate (10×)	PNF001	2×25 mL	It is easy to crystallize at low temperatures, and can be dissolved in 37°C water bath before use. Dilute at 1:10 with freshly prepared ultra-pure water for plate washing.
Anti-CHO:HRP (100×)	PNN002	1×120 µL	Affinity purified sheep antibody conjugated to HRP. Dilute 100 times before use.

TMB Substrate	PND005	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-CHO HCP Microtiter Strips	Store in the bag with desiccant at 2-8℃ for up to 60 days.
Reconstituted CHO HCP Calibration Standard	Store at 2-8℃ for up to 30 days or below -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
- 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

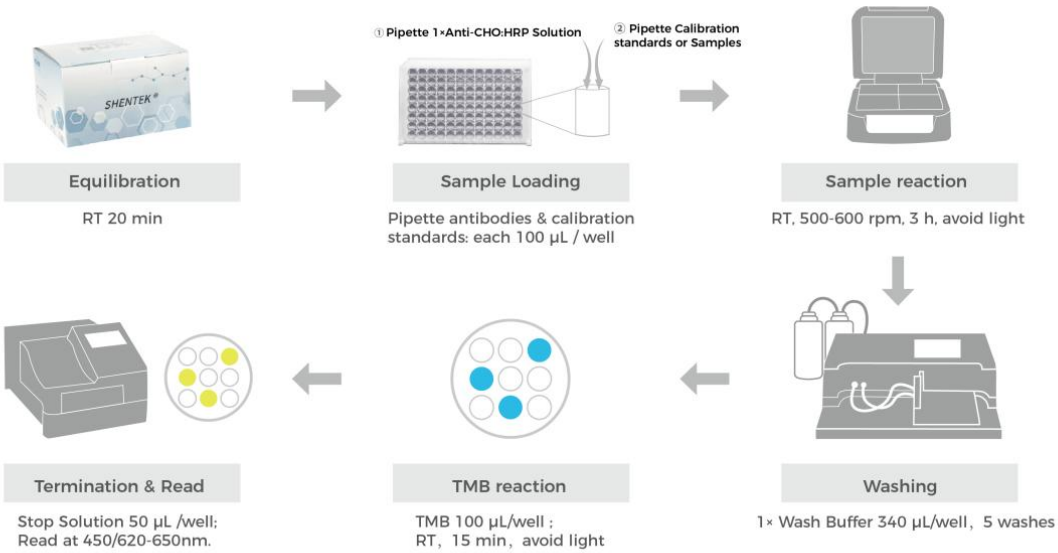


Figure 2. Procedure Flowchart

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

- CHO HCP Calibration Standard Solution: Pipette 500 µL of Reconstitution Solution into the bottle containing CHO 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 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°C until it clears.

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

(3) Preparation of Calibration Standard Solutions

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

Figure 3. Graphic scheme of CHO HCP Calibration Standard Solutions

Table 3. Preparation of CHO HCP Calibration Standard Solutions

Tubes	Dilution procedure	Conc. (ng/mL)
ST1	Dilute the reconstituted CHO HCP Calibration Standard to ST1	128
ST2	500 µL ST1 + 500 µL Diluent	64
ST3	250 µL ST2 + 750 µL Diluent	16
ST4	250 µL ST3 + 750 µL Diluent	4
ST5	500 µL ST4 + 500 µL Diluent	2
ST6	500 µL ST5 + 500 µL Diluent	1
NCS	Diluent	0

(4) Sample Preparation

- Test samples: Cell culture fluid, 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 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-CHO:HRP Solution into each designated well according to the experiment 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.

Table 4. Example of 96-well plate layout

	1	2	3	4	5	6	7	8	9	10	11	12
A	ST1	ST1	ST1		S1	S1	S1					
B	ST2	ST2	ST2		S2	S2	S2					
C	ST3	ST3	ST3		S3	S3	S3					
D	ST4	ST4	ST4		S1+SRC	S1+SRC	S1+SRC					
E	ST5	ST5	ST5		S2+SRC	S2+SRC	S2+SRC					
F	ST6	ST6	ST6		S3+SRC	S3+SRC	S3+SRC					
G												
H	NCS	NCS	NCS									

- ✧ “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 1× Wash Buffer for about 340 µL each 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.

  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.

(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 duplicate 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.

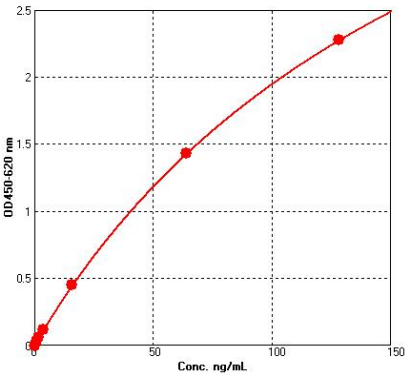
■ Limitations

- For research purposes only; not intended for clinical use.
- Specifically designed for detecting residual protein content from CHO cell line production process.
- 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: 1-128 ng/mL, R^2>0.990.
- LLOQ: 1 ng/mL.
- Specificity: No cross-reactivity with MDCK, Vero, HEK293T, *E.coli*, *P.pastoris* and Sf9 cells.
- Typical calibration curve and results:

Calibration Standards (ng/mL)	Abs. At (450 nm-620 nm)	AVG
128	2.456	2.363
	2.395	
	2.239	
64	1.591	1.519
	1.509	
	1.457	
16	0.554	0.542
	0.548	
	0.524	
4	0.207	0.205
	0.211	
	0.198	
2	0.154	0.148
	0.149	
	0.141	
1	0.122	0.121
	0.119	
	0.120	
0	0.091	0.089
	0.086	
	0.089	



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

A = 5.81069
B = -0.97488
C = 200.99833
D = -0.00435
R^2 = 0.99999

■ 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 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: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.
- ✧ Discard the CHO HCP Calibration Standard and 1×Anti-CHO:HRP due to instability. 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 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 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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