P. pastoris HCP ELISA Kit

(One-step ELISA)

User Guide

PLEASE READ THE DOCUMENT CAREFULLY BEFORE EXPERIMENT

Product No.: 1301313
Version: A/2
For Research Use Only. Not for diagnostic or therapeutic use.

Huzhou Shenke Biotechnology Co., Ltd.

■ Product Name

P. pastoris 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 manufactured with *Pichia pastoris (P. pastoris)* strains, including GS115 and X33.

The product described herein is for research use only and is not intended for diagnostic or therapeutic use.

■ Product Description

This kit is based on the solid-phase enzyme-linked immunosorbent assay (ELISA) with a double-antibody sandwich technique to detect residual host cell proteins (HCPs) from *P. pastoris* cells.

A polyclonal antibody specific to *P. pastoris* HCPs was employed in the assay to capture any remaining HCPs in the sample. The antibody coverage is assessed by the current mainstream method. Both the Calibration Standard (or test sample) and the HRP (Horseradish Peroxidase) labeled with anti-*P. pastoris* HCP antibody were simultaneously added to the microtiter plate 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 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.

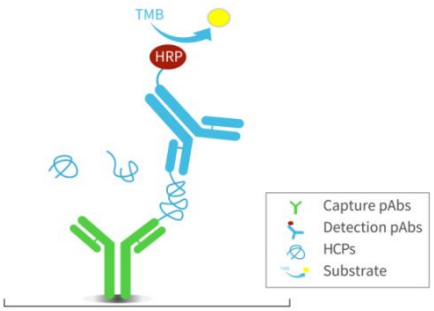


Figure 1. Schematic diagram

■ Kit Contents

Table 1. Kit Components

Reagent	Part No.	Quantity	Note
P. pastoris HCP Calibration Standard	PNB015	2 bottles	Lyophilized powder. Dissolve it with 500 μL Reconstitution Solution (PNC002) and let it stand for about 5 minutes until transparent. Please refer to the bottle label for details.
Anti-*P. pastoris* HCP Microtiter Strips	PNA015	8 well × 12 strips	Strips pre-coated with sheep (*Ovis aries*) anti-*P. pastoris* HCP antibody in a vacuumed bag with desiccant. Seal and store immediately after use.
Reconstitution Solution	PNC002	1 × 1.5 mL	Only used for dissolving *P. pastoris* HCP Calibration Standard (PNB015).
Diluent	PNE006	2 × 25 mL	For dilution of Calibration Standard, Anti-*P. pastoris*:HRP (100×) (PNN009) and samples.
Wash Buffer Concentrate (10×)	PNF001	2 × 25 mL	Dilute 10 times with freshly prepared ultra-pure water for plate washing.
Anti-*P. pastoris*:HRP (100×)	PNN009	1 × 120 μL	HRP-conjugated sheep polyclonal antibody specific for *P. pastoris* HCP. Dilute 100 times before use.
TMB Substrate	PND004	1 × 12 mL	Equilibrate to room temperature (RT) for 20 minutes before use. Seal and keep away from light.
Stop Solution	PNI002	1 × 6 mL	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℃. 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-*P. pastoris* HCP Microtiter Strips	Store in the bag with desiccant at 2-8℃ for up to 60 days.
Reconstituted *P. pastoris* HCP Calibration Standard	For short term use, please store at 2-8℃ for up to 7 days. For long term storage, keep the component below -20℃ for up to 60 days.

■ Materials Required (Not Provided in the Kit)

- 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 pipettes
- Microplate thermoshaker
- Incubator (optional)
- Plate washer (optional)

■ Workflow

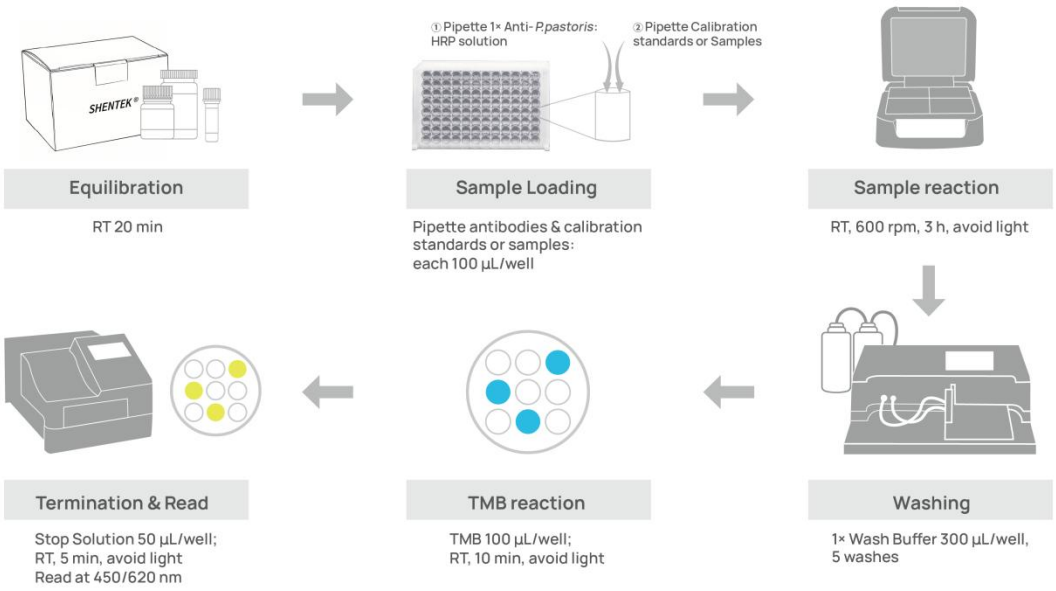


Figure 2. Procedure Flowchart

1. Preparation

(1) Equilibration

- Before use, allow the kit to equilibrate at room temperature for 20 minutes. Return to 2-8℃ after use.
- Take 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

- *P. pastoris* HCP Calibration Standard solution: Pipette 500 µL of Reconstitution Solution (PNC002) into the bottle containing *P. pastoris* HCP Calibration Standard (PNB015). 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 (PNC002) to dissolve the Calibration Standard.*
- 1×Wash Buffer: Dilute the Wash Buffer Concentrate (10×) (PNF001) at 1:10 with ultra-pure water. For example, add 25 mL Wash Buffer Concentrate (10×)

(PNF001) 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×) (PNF001) or Diluent (PNE006) is cloudy or contains precipitates, heat at 37℃ until it clears.*

- 1×Anti-*P. pastoris*:HRP: Prepare the 1×Anti-*P. pastoris*:HRP by diluting the Anti-*P. pastoris*:HRP (100×) (PNN009) with Diluent (PNE006) in a sterile centrifuge tube. Gently mix the solution and use it immediately.

(3) Preparation of Calibration Standard Solutions

- Prepare *P. pastoris* HCP Calibration Standard solutions as shown in Figure 3 and Table 3.

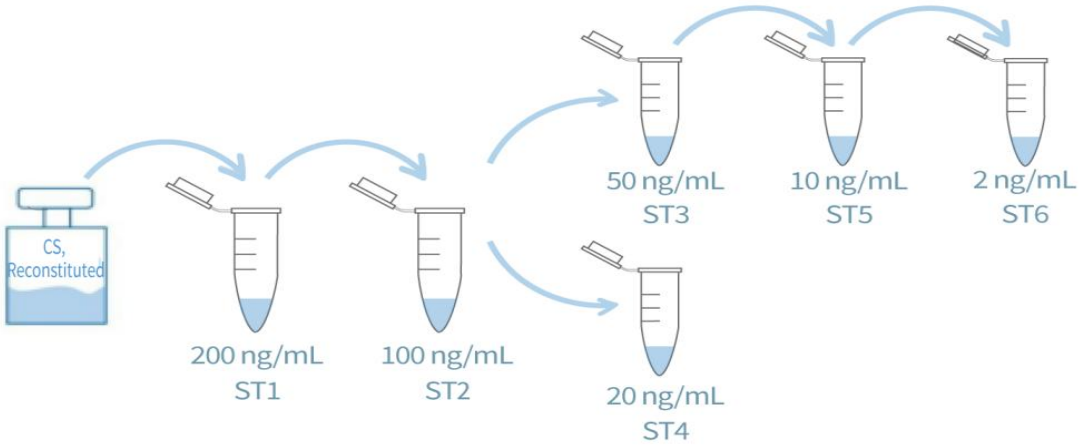


Figure 3. Graphic scheme of *P. pastoris* HCP Calibration Standard solutions

Table 3. Preparation of *P. pastoris* HCP Calibration Standard solutions

Tubes	Dilution Procedure	Conc. (ng/mL)
ST1	Dilute reconstituted *P. pastoris* HCP Calibration Standard to ST1	200
ST2	500 µL ST1 + 500 µL Diluent	100
ST3	400 µL ST2 + 400 µL Diluent	50
ST4	100 µL ST2 + 400 µL Diluent	20
ST5	100 µL ST3 + 400 µL Diluent	10
ST6	100 µL ST5 + 400 µL Diluent	2
NCS	Diluent	0

- Calculate the volume of Diluent (PNE006) to prepare the ST1:

$$\frac{(A\ \mu g \times 1000\ ng/\mu g) \times C\ \mu L}{B\ (500\ \mu L) \times (D\ ng/mL\ /\ 1000\ \mu L/mL)} - C\ \mu L = \text{Diluent volume (X)}$$

- A = Content of Calibration Standard (µg)

- B = Volume of Reconstitution Solution (PNC002, 500 µL)
- C = Volume of reconstituted calibration standard solution for ST1 preparation (*e.g.* 100 µL)
- D = Concentration of ST1 calibration standard solution (200 ng/mL)

For example, the label indicates that each bottle of *P. pastoris* HCP Calibration Standard (PNB015) contains 1.2 µg (A). Add (B) volume (500 µL) of Reconstitution Solution (PNC002) into the bottle for reconstitution. Then take (C) volume (recommended 100 µL) of the reconstituted solution and add 1100 µL (X) of Diluent (PNE006) to prepare a ST1 calibration standard solution with a concentration of 200 ng/mL(D).

$$\frac{(1.2\ \mu g \times 1000\ ng/\mu g) \times 100\ \mu L}{500\ \mu L \times (200\ ng/mL / 1000\ \mu L/mL)} - 100\ \mu L = 1100\ \mu L$$

(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-thaw cycles. For long-term storage, -70°C is recommended to avoid degradation.
- Dilute the samples with a suitable diluent (PNE006) to achieve a proper range of HCP concentration within the calibration curve.
- For the first use, a method validation is recommended 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 100 µL of 1×Anti-P. pastoris:HRP solution into each designated well according to the experimental design.
- Pipette 100 µL of Calibration Standard solutions, controls 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) Substrate Incubation

- Equilibrate the TMB substrate (PND004) 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 (PND004) into the wells, and incubate at RT for 10 minutes, and protect from light.

Note: *Do not use Sealing Film (PNK001) for this step.*

(3) Termination

- Add 50 µL of Stop Solution (PNI002) into each well.

Note: *The adding sequence should be the same as the adding sequence of the TMB Substrate (PND004). 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, and protect from light.

(4) Read

- Read absorbance at 450 nm/620-650 nm.

3. Calculation and Analysis

- The $OD_{450\ nm}$ value of each well should be calculated by subtracting their respective long wavelength, as of $OD_{620\ nm}$ in this case. If the microplate reader is not equipped with long wavelength measurement, this step can be omitted.
- The $OD_{450\text{-}620\text{-}N}$ value of calibration curve fitting points and samples should be calculated by subtracting the $OD_{450\text{-}620}$ of NCS, then take the average value of replicates.
- Perform a 4-parameter logistic regression model using the subtracted Calibration Standard concentration values to obtain a calibration curve equation. The data analysis software for calibration curve fitting could be the built-in software from microplate reader. If not, we recommend to use professional standard curve software such as Curve Expert, ELISA Calc, and so on.
- Substitute the average OD value of the samples into the equation to calculate the sample concentration.

■ Limitations

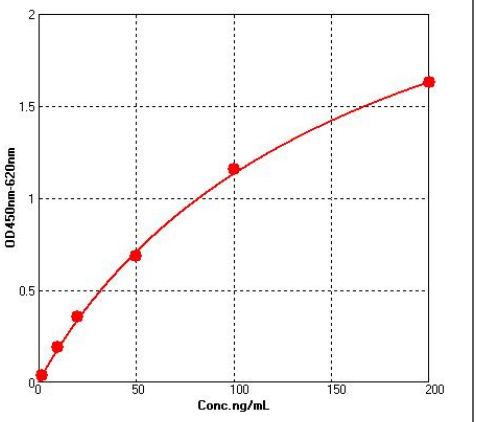
- Recommend sample pH between 6.5 and 8.5, and measurements may be compromised if the sample pH is out of range.
- Components in sample buffer or formulation should not be produced by P. pastoris. Otherwise, abnormal results may occur due to contamination.
- Protein enzymes in sample should be inactivated by appropriate methods. Otherwise, abnormal results may occur due to enzymatic digestion.

■ Assay Performance

- Linearity& Range: 2-200 ng/mL, $R2 \geq 0.990$
- QL: 2 ng/mL
- Specificity: No cross-reactivity with HCPs from E. coli, CHO and Sf9 cells, cross-reactivity less than 1% with HCPs from *Saccharomyces cerevisiae* and *Hansenula polymorpha* cells.

- Typical calibration curve results for reference:

Calibration Standards (ng/mL)	Abs. at (450 nm-620 nm)			AVG
200	1.748	1.681	1.684	1.704
100	1.244	1.225	1.229	1.233
50	0.763	0.768	0.753	0.761
20	0.434	0.444	0.423	0.434
10	0.271	0.270	0.270	0.270
2	0.117	0.111	0.115	0.114
0	0.079	0.078	0.076	0.078



■ 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 (PNI002) 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-*P. pastoris*:HRP(100×) (PNN009) 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.
- ✧ *P. pastoris* HCP Calibration Standard solutions and anti-*P. pastoris* HCP Antibody solution are recommended for single use due to instability issue. Prepare freshly before each experiment.
- ✧ TMB Substrate (PND004) 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.
- ✧ For more stable results, reading should be completed within 10 minutes after termination, but no more than 30 minutes.
- ✧ 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 equipment, including micropipettes and centrifuge	Clean the equipment with 75% ethanol before experiment
	Environmental 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 (PNK001) and remove it carefully to prevent splashing.

If you have any other questions, please contact us for technical support.

■ References

- USP <1132> Residual Host Cell Protein Measurement in Biopharmaceuticals
- USP<1103> Immunological Test Methods Enzyme-Linked Immunosorbent Assay (Elisa)
- Ph.Eur. <2.6.34> Host-Cell Protein Assays
- ChP<9101> Guidance for Analytical Procedures Validation
- JP <G3-9-172> Host Cell Protein Assay

Effective date: 01 Mar. 2026

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