NEED HELP?

User Manual

Disclaimer: Products are intended for research use only

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P.pastoris HCP ELISA Kit

(One-step ELISA)

User Guide

PLEASE READ THE DOCUMENT CAREFULLY BEFORE EXPERIMENT

Product No.: 1301313
Version: A/1
For Research Use Only

Biofargo, Inc.

■ 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 such as GS115 and X33 and so on.

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

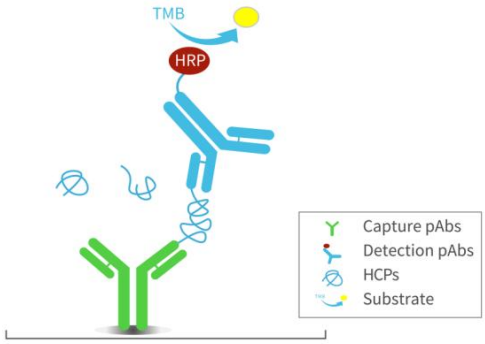


Figure 1. Schematic diagram

■ Kit Contents

Table 1. Kit Components

Reagent	Part No.	Quantity	Note
P.pastoris HCP Calibration Standard	PNB015	2 bottles	Lyophilized powder. Dissolve it with the reconstitution solution (500 μL), and let it stand for about 5 minutes until transparent. Please refer to the details on the label of the tube.
Anti-*P.pastoris* HCP Microtiter Strips	PNA015	8 well ×12 strips	Strips pre-coated with sheep 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.
Diluent	PNE006	2×25 mL	For dilution of Calibration Standard, Anti-*P.pastoris*:HRP(100×) and samples.
Wash Buffer Concentrate (10×)	PNF001	2×25 mL	Dilute 10 times with freshly prepared ultra-pure water to obtain 1×Wash Buffer Solution.
Anti-*P.pastoris* :HRP (100×)	PNN009	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	Sealed and keep away from light. Equilibrate to room temperature (RT) for 20 minutes before use.
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℃. 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 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

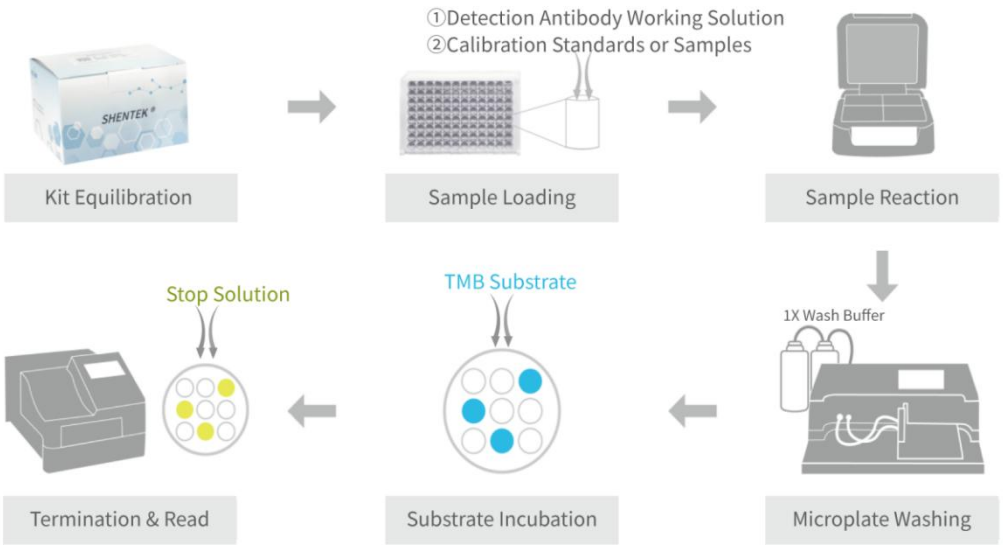


Figure 2. Procedure Flowchart

1. Preparation

(1) Equilibration

- Before use, allow the kit to equilibrate at room temperature for 20 minutes. 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 into the bottle containing *P.pastoris* HCP Calibration Standard. Gently invert to mix and let it stand for 5 minutes. Save the remaining solution under the recommended condition.

  Note: If two or more vials of calibration standards are applie, combined all after reconstituted, and mix gently before use.
- 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-*P.pastoris* :HRP: Prepare the 1×Anti-*P.pastoris* :HRP by diluting the Anti-*P.pastoris* :HRP(100×) with Diluent in a sterile centrifuge tube. Prepare fresh 1×Anti-*P.pastoris* :HRP, mix gently and use immediately.

(3) Preparation of Calibration Standard Solutions

- Prepare *P.pastoris* HCP Calibration Standard Solutions as showed in Fig 3 and Table 3.

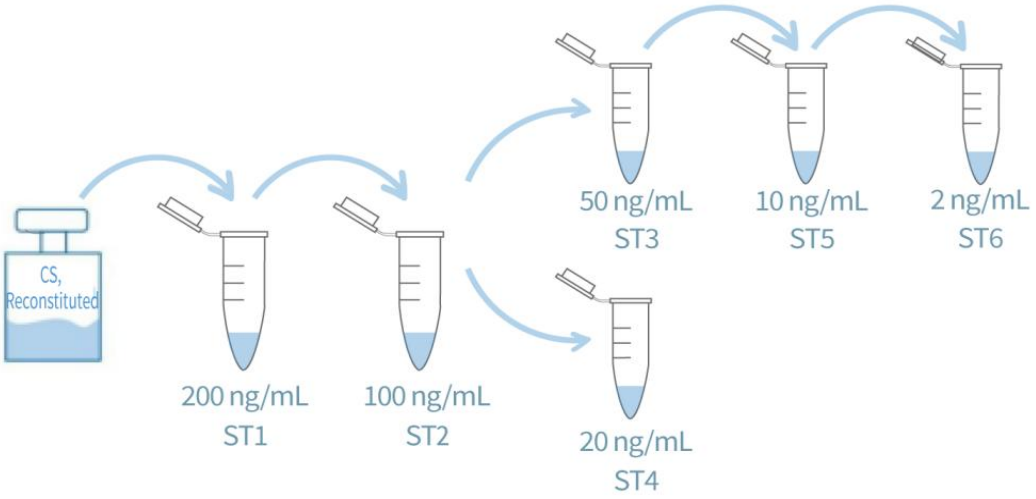


Figure 3.Graphic scheme of *P.pastoris* HCP Calibration Standard Solutions

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

Tubes	Dilution Procedure	Conc. (ng/mL)
ST1	Dilute reconstituted *P.pastoris* HCP Calibration Standard (CS) 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

(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 to achieve a proper range of HCP con centration within the calibration curve. Typically, 10-folds or more are recomme nded.
- 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 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 for 20 min 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 10 minutes, and protect from light.

  Note: Do not use sealing film during this step.

(3) Termination

- Add 50 μL of Stop Solution into each well.

  Note: The order of adding stop solution should be the same as the order of adding the TMB solution. While adding samples, suspend the tips above the liquid 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 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.

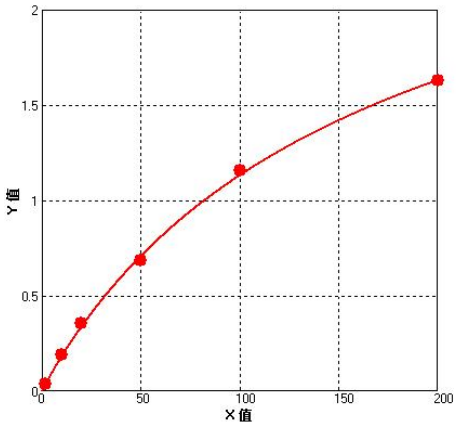
■ Limitations

- This product is intended for research use only but not for clinical applications.
- The samples pH should be between 6.5 and 8.5. Beyond this range may cause abnormal results.
- 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, 4-PL, $R^2 \geq 0.990$
- LLOQ: 2 ng/mL
- Specificity: No cross-reactivity with *E.coli*, CHO and Sf9 cells, cross-reactivity less than 1% with *Saccharomyces cerevisiae* and *Hansenula polymorpha*.
- Typical calibration curve results for reference

Calibration Standards(ng/mL)	Abs. at 450nm-620nm			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.
- ✧ 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 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×) 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 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 terminati on, 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
	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

- ICH. M10. Bioanalytical Method Validation And Study Sample Analysis
- FDA. Bioanalytical Method Validation
- ChP<9012>Guidance of Quantitative Method Validation for Biological Samples
- USP<1103> Immunological Test Methods — Enzyme-Linked Immunosorbent Assay (Elisa)
- JP<G3-11-171> Enzyme-linked Immunosorbent Assay (ELISA)
- ChP<3429>Immunochemical Method
- USP<1132> Residual Host Cell Protein Measurement in Biopharmaceutical
- EP<2.6.34>HOST-CELL PROTEIN ASSAYS
- JP<G3-9-172> Host Cell Protein Assay

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Support & Contact

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