# *Pichia pastoris* Host Cell Protein ELISA Kit, G3

# (Applicable to Strain X-33 and Derivatives)

**96 Wells**

**Catalogue Number: PH-E0021-3**

**ELISA Kit for the quantitative Measurement of *Pichia pastoris* HCP Residues in Culture Supernatants, Protein Purification Process, and End-Product**

**FOR RESEARCH, DEVELOPMENT AND MANUFACTURING USE ONLY!**
**NOT FOR THERAPEUTIC OR DIAGNOSTIC APPLICATIONS!**
**PLEASE READ THROUGH ENTIRE PROCEDURE BEFORE BEGINNING!**

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## 1. APPLICATION

This kit is intended for the quantitative detection of host cell protein (HCP) residues in biopharmaceuticals produced by *Pichia pastoris* (Strain X-33 and Derivatives) expression systems.

## 2. PRINCIPLE OF THE ASSAY

This ELISA kit is applied to the solid phase sandwich Enzyme-Linked Immunosorbent Assay. The microplate has been pre-coated with a capture antibody. The antigen to be measured and the HRP-conjugated detection antibody are then added to the microplate wells sequentially to form a capture antibody-antigen-detection antibody complex. After washing, the conjugates uninvolved in the reaction are removed, and then a TMB substrate solution is added to the wells to incubate. TMB substrate solution turns blue after the oxidation of HRP and finally turns yellow immediately after adding the stop solution. The color of the TMB substrate positively correlated with the antigen bound in the initial steps. Measure the absorbance (OD value) at 450nm using a microplate reader, and create a standard curve along with the corresponding concentrations. Then, by inputting the OD values of the samples into the standard curve equation, calculate the concentration of the target protein in the sample.

## 3. KITS' ADVANCEMENT

1) High Coverage: The antibodies exhibit strong recognition of host cell proteins (HCPs), with a coverage rate exceeding 80% and consistent performance across manufacturing processes.
2) High Antibody Titers: The antibodies used in the reagent kit are determined by ELISA, and the results indicate a titer of at least $10^6$.
3) High Sensitivity: Serum antibody purification employs affinity purification to remove non-specific antibodies to the greatest extent.
4) High Stability: The production process uses a broad-spectrum protein stabilizer and microplate processing technology to enhance the stability and repeatability of the standard and microplate result.
5) Optimal Diluent Buffer: The sample diluent has been optimized to accommodate the different buffer systems used at various stages of antibody purification. This significantly reduces

nonspecific binding during sample analysis, improves dilution linearity and recovery rates, and maintains an extremely low background signal, facilitating accurate detection of the target sample concentration.

## 4. MATERIALS (Note: Store at 2-8°C)

| | Reagents | Specification | Quantity |
|---|---|---|---|
| 1 | Pre-Coated Microplate (Detachable) | 96 wells | 1 plate (Keep Sealed) |
| 2 | Standard (Stock Solution) -500μg/mL | 50μL | 1 tube |
| 3 | Detection antibody (100×) | 150μL | 1 tube |
| 4 | TMB Substrates | 10mL | 1 vial (Avoid Light) |
| 5 | Stop Solution | 10mL | 1 vial |
| 6 | Wash Solution (20×) | 10mL | 5 vial |
| 7 | Diluent Buffer (10×) | 10mL | 1 vial |
| 8 | Plate Sealer | | 4 pieces |
| 9 | Instruction Manual | | 1 |

## 5. EQUIPMENT REQUIRED BUT NOT PROVIDED

1) 10-1000μL pipettor
2) Multichannel pipettor
3) 1L sterilized deionized water or ultrapure water
4) Sterilized EP tubes
5) Absorbent Paper
6) Microplate reader
7) High-speed centrifuge
8) Mini Centrifuge
9) Microplate washer or washing bottles
10) Data analysis and graphing software

## 6. PREPARATION BEFORE ASSAY

Please read the kit instruction manual carefully before use. The reaction should be carried out at

room temperature.

## 7. REAGENT PREPARATION

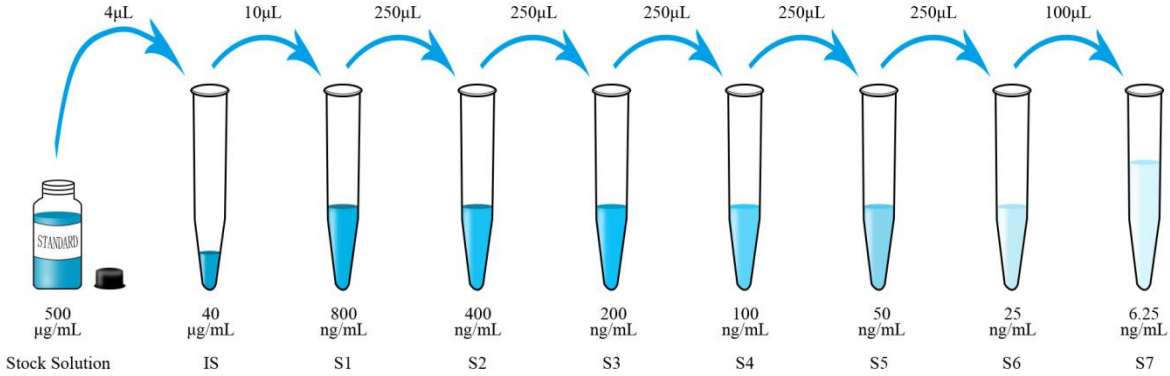
1) Place all kits' components at room temperature for 30mins before using.
2) The preparation of Wash Solution (1×): Add the contents of five 10mL of Wash Solution (20×) into 950mL of deionized water or ultrapure water to prepare 1000mL of Wash Solution (1×). If crystals have formed in the concentrate, allow it to reach room temperature and mix gently until the crystals are completely dissolved.
3) The preparation of Diluent Buffer (1×): Dilute 10mL of Diluent Buffer (10×) with 90mL of deionized water or ultrapure water to prepare 100mL of Diluent Buffer (1×) . If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. The buffer is used as the Diluent Buffer for standards, samples and detection antibodies.

## 8. ASSAY PROCEDURE

1) Remove the microplate from the foil bag, detach the desired number of strips from the plate, immediately reseal the remaining strips and store at 4°C. Then put the ready-for-use strips on a clean microplate frame, and start the experiment after the strips returned to room temperature (Note: The microplate frame can be reused). The standard may stick to the tube wall due to transport turbulence. Before using, gently shake it or centrifuge for about 2 seconds.
2) Prepare Standards: When preparing the standards, label 8 tubes, IS and S1-S7. Add a certain volume of Diluent Buffer (1×): IS (46μL), S1 (490μL), S2 to S6 (each 250μL), S7(300μL). Add 4μL of the Standard (Stock Solution-500μg/mL) into the tube labeled as IS. Mix gently by pipetting twice and inverting-do not vortex or mix vigorously (same for all subsequent steps). Next, take 10μL IS (40μg/mL), and add it to tube S1. Shake well and then pipette 250μL Standard solution (800ng/mL) from S1 to S2. And then produce a 2-fold dilution series until S6. Finally, transfer 100μL from S6 to S7 and mix gently. (see below). Secure the desired plate in the holder then add 100μL of diluted standards (S1-S7) or 100μL (diluted or undiluted) samples to appropriate wells.

For the Blank Control test, we recommend add 100μL Diluent Buffer (1×) to the well.

**NOTE:** There are 7 points of diluted standards, S1: 800ng/mL, S2: 400ng/mL, S3: 200ng/mL, S4: 100ng/mL, S5: 50ng/mL, S6: 25ng/mL, S7:6.25ng/mL.




3) Cover the plate with sealer, put it on the oscillator to mix and incubate for 90 mins at room temperature.

4) Automated Washing:

- Put 1000mL Wash Solution (1×) into the washing bottle of the automated microplate washer for standby.
- Take out the microplate, and discard the incubation mixture. And then wash the microplate with the automated microplate washer for 5 times.
- After washing, invert plate and blot dry by hitting the plate onto absorbent paper or paper towels until there is no moisture appears.

Or Manual Washing:

- Put 1000mL Wash Solution (1×) into the washing bottle for standby.
- Take out the microplate, and discard the incubation mixture. And then invert plate and blot dry by hitting the plate onto absorbent paper or paper towels until there is no moisture appears.
- Fill each well with 300μL Wash Solution (1×) by a muti-channel pipette, let stand for 20 seconds, then discard the contents, invert plate, and blot dry by hitting the plate onto absorbent paper. Repeat this washing step for 5 times. Note: There should be no moisture appears in the fifth washing step.

5) Prepare Detection Antibody: Extract 100μL of Detection antibody (100×), and add it into 10mL

Diluent Buffer to reach its working concentration (1×) and mix gently. Add 100μL of above diluted detection antibody to each well, cover the plate with a sealer, and put it on the oscillator to mix and incubate for 90mins at room temperature.

6) Washing Step: Repeat the same procedure as step 4.
7) Add 100μL TMB substrate to each well. Cover the plate with a sealer, incubate at room temperature for about 15mins. If the color is light, the reaction time can be extended appropriately, but not more than 30min.
8) Add 50μL of Stop Solution to each well to stop the reaction.
9) Run the microplate reader and conduct measurement at 450nm.
10) Data analysis: Four parameter curve fitting is recommended.

## 9. NOTES

1) **Sample Preparation**
   a) After collection, the samples should be aliquoted and stored at -20°C (less than 3 months) or -80°C (less than 6 months) to maintain protein activity, avoid contamination and multiple freeze-thaw cycles. If samples are to be run within 24 hours, they may be stored at 2- 8°C.
   b) Ensure the frozen samples are completely thawed before use (do not thaw by heating). Mix thoroughly using a pipette or vortex mixer. If precipitates are present in the sample, they may interfere with the ELISA results; centrifugation is recommended to remove the precipitates.
   c) Some chemical solvents may interfere with this experiment, such as SDS, Triton, etc.
   d) It is recommended that all standards and samples be run in duplicate. Please gently mix the liquid after adding standards and samples, and confirm there's no bubble inside.
   e) The process of target protein purification is often accompanied by complex buffer solutions. To exclude matrix effect, it is recommended to perform spike recovery test when using different buffers for the first time. An acceptable spike recovery range is 80-120%. High salt, low PH, polysaccharide, organic solvents, and detergents can result in lower recovery rates. The common practice is to add Standard S1(800ng/mL)/Diluent Buffer into a buffer

solution in a 1:3 volume ratio(e.g. add 25μL of standard S1(800ng/mL)/Diluent Buffer to 75μL of the buffer solution). The recovery rate is calculated by subtracting the background concentration without the S1 from the concentration with S1, then dividing by the theoretical concentration.

**2) Experimental Operation**

a) Do not mix or interchange different ELISA kits or same ELISA kit but with different lot.

b) To avoid cross-contamination, use disposable sterilized tips during the experiment.

c) Total dispensing time for addition of reagents or samples to the plate should not exceed 10 minutes. If there are too many samples, a multichannel pipettor is recommended.

d) Incomplete washing will adversely affect the test outcome. All washing must be performed with Wash Solution provided. All residual wash liquid must be drained from the wells by efficient aspiration or by decantation followed by tapping the plate forcefully on absorbent paper. Never insert absorbent paper directly into the wells. Do not directly touch the bottom of the microplate. If it is covered with fingerprints or stains, it will affect the measurement.

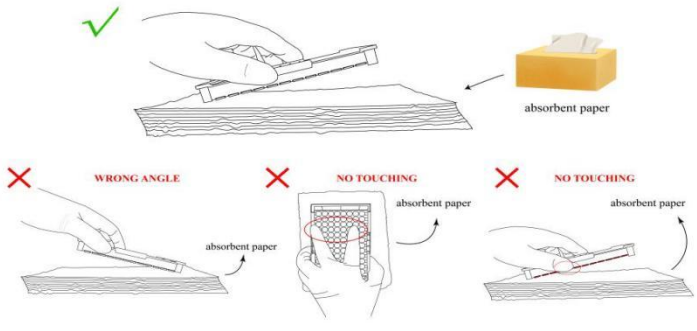
e) TMB is easily contaminated. Please protect it from light and metal.

f) Monitor the reaction time. After adding TMB substrate, observe the color change of reaction wells at regular intervals (for example, about 10 minutes). If the color turns too deep, add Stop Solution in advance to terminate the reaction.

g) The Stop Solution provided with this kit is diluted sulfuric acid. Protective gloves, clothes, and face protection should be worn.

h) The coefficient of determination of the standard curve should be $R^2 \geqslant 0.95$.

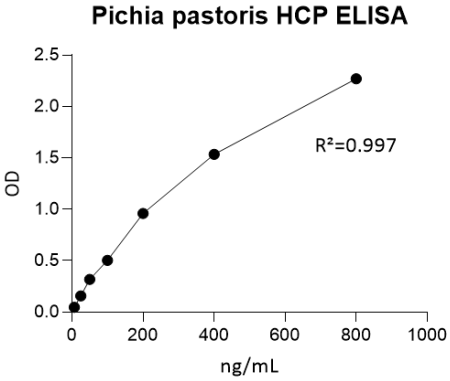
i) Schematic diagram of tapping the plate:



## 10. CALCULATION OF RESULTS

1) Average the duplicate readings for each standard and sample.
2) Construct a standard curve by plotting the average O.D. for each standard on the vertical (Y) axis against the concentration on the horizontal (X) axis, and generate a four paramater logistic (4-PL) curve-fit.
3) Calculate the concentration of samples corresponding to the mean absorbance from the standard curve.
4) Standard curve is for demonstration only.

### APPENDIX 1: EXAMPLE OF STANDARD CURVE




| ng/mL | OD450 |
|---|---|
| 800 | 2.270 |
| 400 | 1.534 |
| 200 | 0.959 |
| 100 | 0.504 |
| 50 | 0.317 |
| 25 | 0.158 |
| 6.25 | 0.046 |

## 11. QUALITY CONTROL

1) Sensitivity:

   LOD: 1.56 ng/mL

   LOQ: 6.25 ng/mL

2) Precision:

   Intra Variation%: 7-11

   Inter Variation%: 1.1-5.8

3) Linearity:

| Diluent ratio | Range % |
|---|---|
| 1:2 | 98.2-103.5 |
| 1:4 | 95.2-102.6 |

| | |
|---|---|
| 1:8 | 98.2-105.3 |
| 1:16 | 95.3-104.1 |
| 1:32 | 97.3-103.4 |
| 1:64 | 88.8-103.2 |

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