Pichia Yeast Host Cell Protein ELISA Kit, G2

96 Wells

Catalogue Number: PH-E0021-1

Valid Period: 6 months

ELISA Kit for the quantitative Measurement of Pichia Yeast 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!

CONTENTS

1. APPLICATION

The kit is intended for the quantitative determination of host cell protein residues in Protein therapeutics expressed by Pichia Yeast expression systems.

2. BACKGROUND

Pichia Yeast, commonly is widely utilized as an expression of therapeutic proteins. The process of expressing target proteins, undergoing concomitant apoptosis, which release up to a thousand of host proteins into cell culture medium after the break of cells. These host proteins exhibit strong immunogenicity, which can lead to adverse toxicity or immune reactions, jeopardizing product safety and quality, and causing potential biological contamination. One of the aims of downstream processes in the production of biological medicinal products is to remove these potential hazards. Therefore, it is essential to minimize the residual levels of host cell proteins (HCP), and in the downstream purification process development, a scientifically sound method for determining the concentration of HCP in finished or semi-finished products is necessary. Enzyme-linked immunosorbent assay (ELISA) has a high sensitivity and is therefore designated as the gold standard for HCP detection by regulatory agencies.

3. 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.

4. KITS' ADVANCEMENT

1) High Coverage: The capture and detection antibody are both derived from rabbit, which have strong recognition of host-cell proteins (HCPs). Moreover, there is little individual variation within this species, ensuring high comparability and process stability.
2) High Antibody Titers: The antibodies used in the reagent kit are tested using the indirect method with 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: Using an optimized dilution solution can reduce non-specific adsorption during the sample detection process, resulting in very low background coloration that facilitates the observation of the concentration of the samples.

5. MATERIALS (Note: Storage at 2-8℃)

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

6. EQUIPMENT REQUIRED BUT NOT PROVIDED

1) 10-1000 µl pipettor and disposable sterilized tips

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

7. SPECIMEN COLLECTION AND STORAGE

Culture Supernatant -Centrifuge them at 1000×g (or 3000 rpm) for 15 minutes, and collect the supernatant for immediate assay or store samples in aliquots at -20°C or -80°C.

NOTE:

- Samples should be aliquoted and must be stored at -20°C (less than 3 months) or -80°C (less than 6 months) to avoid loss of bioactivity and contamination. If samples are to be run within 24 hours, they may be stored at 2- 8°C. Avoid repeated freeze-thaw cycles.
- Some chemical solvents may interfere with this experiment, such as SDS, Triton, etc.
- The precipitates in the sample solution can interfere with ELISA. Make sure to centrifuge and remove them.
- Do not use heat-treated specimens.

8. REAGENT PREPARATION

1) Place all kits' components at room temperature for 30mins before using.
2) The preparation of Wash Solution (1×): Dilute 10 mL of Wash Solution (100×) with 990 mL of deionized water or ultrapure water to prepare 1000 mL of Wash Solution (1×). If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved.
3) The preparation of Diluent Buffer: Dilute 10 mL of Diluent Buffer (10×) with 90 mL of

deionized water or ultrapure water to prepare 100 mL 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.

9. ASSAY PROCEDURE

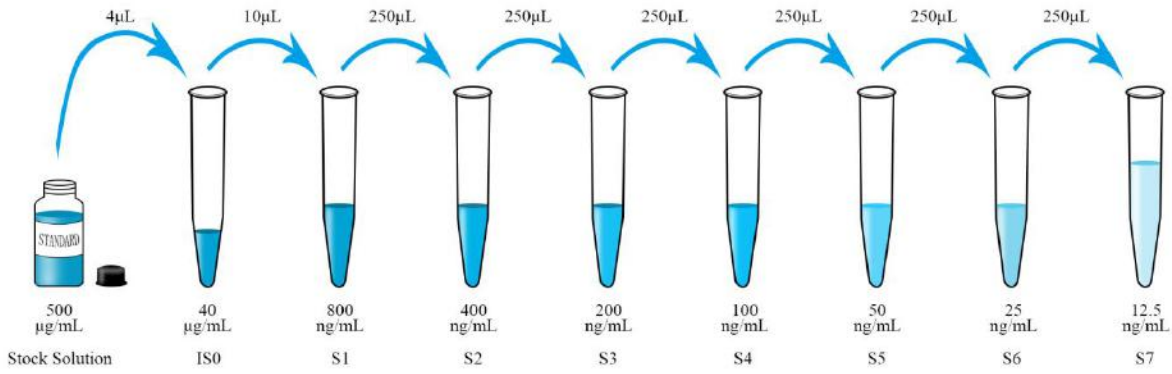
1) Remove the microplate from the foil bag, detach the desired number of strips from the plate, immediately reseal the remaining strips and store at 4°C. Then put the ready-for-use strips on a clean microplate frame, and start the experiment after the strips returned to room temperature (Note: The microplate 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 IS0, S1-S7 and add a certain volume of diluent: IS0 (46ul), S1 (490ul), S2 to S7 (each 250ul).

   Add 4ul of the Standard (Stock Solution-500ug/ml) into the tube labeled as IS0. Shake well and then take 10 ul IS0 (40ul/mL), and add it to tube S1. Shake well and then pipette 250ul Standard solution (800ng/ml) from S1 to S2. And then produce a 2-fold dilution series until S7 (see below). Secure the desired plate in the holder then add 100ul of diluted standards (S1-S7) or 100ul (diluted or undiluted) samples to appropriate wells.

   For the Blank Control test, we recommend add 100ul Diluent Buffer 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, S7:12.5ng/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 1000 ml 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 1000 ml 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 300ul 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 100ul of Detection antibody (100×), and add it into 9.9 ml Diluent Buffer to reach its working concentration (1×) and mix gently. Add 100ul 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 90 mins at room temperature.

6) Washing Step: Repeat the same procedure as step 4.

7) Add 100ul TMB substrate to each well. Cover the plate with a sealer, incubate at room temperature for about 15 mins. If the color is light, the reaction time can be extended appropriately, but not more than 30min.

8) Add 50ul 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.

10. NOTES

1) **Sample Preparation**

a) After collection, the samples should be aliquoted and stored at -20℃ (less than 3 months) or -80℃ (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℃.

b) Samples should be frozen and aliquoted if not analyzed shortly after collection, avoid multiple freeze-thaw cycles. Thaw the frozen samples completely and mix well by pipettor or Vortex, centrifuge to remove flocculent insoluble substances before use.

c) 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.

d) The process of 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. 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 1:4 volume ratio(e.g. add 20ul of standard S1(800ng/ml)/Diluent Buffer to 80ul 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.

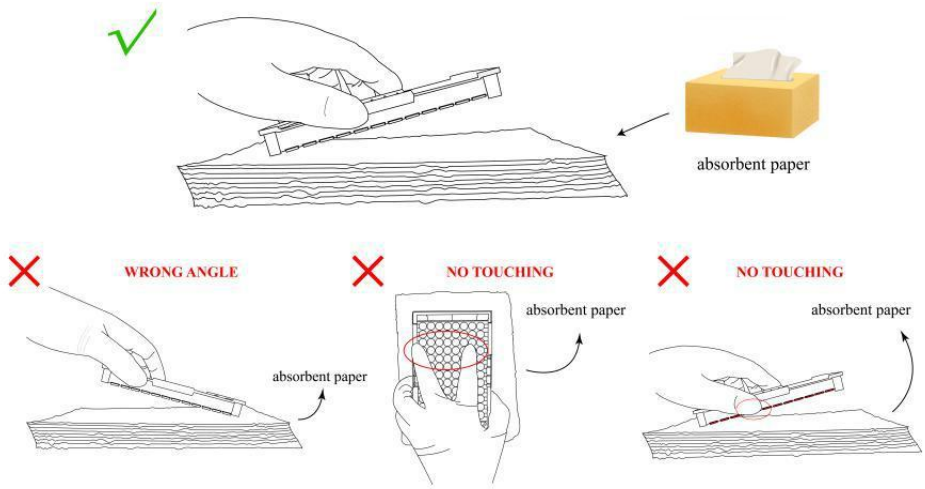
e) TMB is easily contaminated. Please protect it from light and metal.

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:



11. CALCULATION OF RESULTS

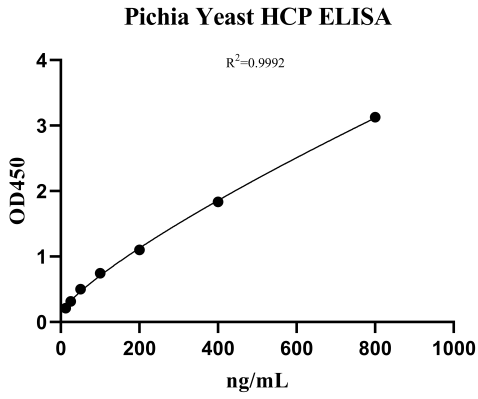
1) Average the duplicate readings for each standard and sample.

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	3.127
400	1.836
200	1.102
100	0.745
50	0.501
25	0.315
12.5	0.212

12. QUALITY CONTROL

1) Intra Variation%: 7-11

2) 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

4) Sensitivity:

LOD: 1.56 ng/ml

LOQ: 12.5 ng/ml

13. CONTACT US

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