Version 18-11/24

User's Manual

P. pastoris HCP ELISA Kit

REF **DEIA-NS2410-4**

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RUO

This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

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PRODUCT INFORMATION

Intended Use

Pichia pastoris HCP Residue Detection Kit (One-step ELISA) is suitable for the quantitative detection of host residual proteins in biological products produced by Pichia pastoris strains.

Principles of Testing

This kit is based on the ELISA method and uses a double antibody sandwich method to quantitatively detect the residual HCPs of Pichia in the sample to be tested.

This analytical method adds calibrators or samples to be tested and HRP-labeled anti-Pichia HCPs sheep polyclonal antibodies to the ELISA plate pre-coated with anti-Pichia HCPs sheep polyclonal antibodies for co-incubation; after washing, TMB substrate is added for color development, and finally the enzyme catalytic reaction is terminated with a stop solution. The absorbance is measured at a wavelength of 450 nm using an ELISA reader. The absorbance is positively correlated with the concentration of HCPs in the calibrator or sample to be tested. The concentration of Pichia HCPs in the sample to be tested can be calculated by fitting the dose-response curve of the calibrator.

This kit does not require special treatment of the sample to be tested, and can be used directly after the applicability is verified by a suitable dilution ratio. This kit has few operating steps, is fast, has strong detection specificity, and has stable and reliable performance.

Reagents And Materials Provided

1. Pre-coated ELISA plate: 8 wells × 12 strips
2. Pichia pastoris HCP calibrator: 2 bottles, lyophilized powder. Accurately measure 500 µL of calibrator reconstitution solution, dissolve, and let stand for 5-10 min. The solution should be clear and transparent, with no visible insoluble matter. It should be stored away from light. For product information, please refer to the label.
3. Calibrator reconstitution solution: 1.5 mL × 1 tube. For calibration only.
4. Sample diluent: 25 mL × 2 bottles.
5. Concentrated wash buffer (10×): 25 mL × 2 bottles
6. Pichia pastoris HCP enzyme-labeled antibody (100×): 120 µL × 1 tube.
7. TMB colorimetric solution: 12 mL × 1 bottle
8. Stop solution: 6 mL × 1 bottle

Materials Required But Not Supplied

Sterile centrifuge tubes for dilution

Pipette tips

Absorbent paper for patting the ELISA plate dry

Pipette well

ELISA reader (capable of measuring absorbance at a single wavelength between 450 nm and 620-650 nm)

Single-channel or multi-channel micropipette

Microplate thermostat oscillator

Incubator (optional)

Microplate washer (optional)

Storage

Unopened kit should be stored at 2-8°C. The validity period is 12 months.

Specimen Collection And Preparation

1. Samples: including upstream expression, downstream purification process samples and stock solutions, finished preparations, etc. Samples should be clear and transparent, and insoluble matter should be removed by centrifugation or filtration.
2. Processing: The sample to be tested is diluted with diluent by an appropriate multiple according to its estimated HCPs concentration, so that its detection value falls within the quantitative range of the calibration curve. It is recommended to select a sample dilution multiple of more than 10 times to ensure that the diluent has a normal effect.
3. For the first use or when the HCPs content of the sample is unknown, it is strongly recommended to verify the sample suitability and determine the appropriate sample dilution multiple for better subsequent routine testing.

Reagent Preparation

(I) Preparation before the experiment

1. Take out the pre-coated ELISA plate and equilibrate it at room temperature for about 20 minutes. The remaining reagents must be taken out in advance before use and equilibrated at room temperature; immediately put them back at 2-8 °C for storage after use.
2. Calculate the number of wells required according to the number of test samples, take out the corresponding number of pre-coated ELISA strips, seal the remaining strips together with the desiccant in a ziplock bag, put them back into the reagent kit, store them in a refrigerator at 2-8 °C, and use them up within the validity period.

Note: Some wells of the ELISA plate may have crystals, which is a normal phenomenon and does not require special treatment.

Note: Room temperature refers to 25 °C ± 3 °C.

(II) Reagent preparation

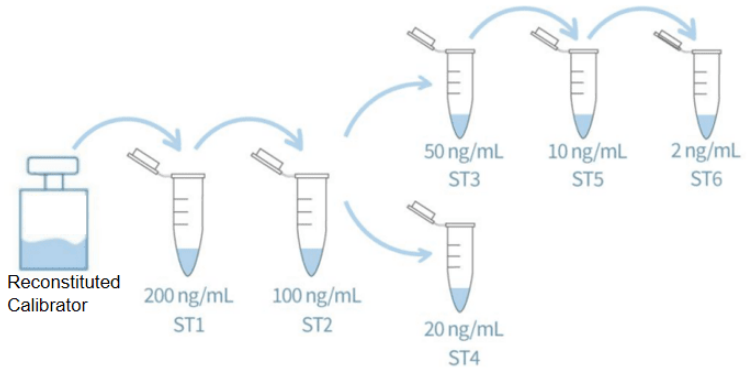
1. Dissolution of Pichia pastoris HCP calibrator: Accurately measure 500 µL of the calibrator reconstitution solution, add it to the vial, gently invert to mix, and let it stand for 5-10 minutes to obtain the reconstituted calibrator.

Note: Calculate the concentration of the reconstituted calibrator according to the label information before performing gradient dilution. If you need to use multiple bottles of calibrators at the same time, please dissolve them separately, transfer them, and combine them into a 1.5 mL sterile centrifuge tube. Shake and mix before use.

2. 1×Wash buffer: Concentrated wash buffer (10×) is diluted 10 times with ultrapure water. For example, take 25 mL Concentrated wash buffer (10×) and add 225 mL ultrapure water to mix, which is 1×Wash buffer for washing the plate. It is recommended to prepare and use it immediately. If the amount of 1×Wash buffer reagent is insufficient due to washing with a plate washer, you can purchase Wash buffer with the same product number separately.

Note: Take out the Concentrated wash buffer (10×) and diluent for observation. If there is crystallization, it is normal. Incubate at 37 °C until it is completely dissolved.

3. Preparation of detection antibody: Dilute it 100 times with diluent in a sterile centrifuge tube, gently invert and mix, which is 1×Pichia pastoris HCP enzyme-labeled antibody. Prepare an appropriate volume to ensure that there is sufficient margin when adding liquid. Prepare and use immediately.
4. Prepare the calibration curve: Perform gradient dilution of the calibration product according to the diagram and table.



Calibration curve samples	Dilution method	Concentration(ng/mL)
ST1	Reconstitute the calibrator with diluent to the ST1 concentration.	200
ST2	500μLST1+500μL Dilution	100
ST3	400μLST2+400μLDilution	50
ST4	100μLST2+400μLDilution	20
ST5	100μLST3+400μLDilution	10
ST6	100μLST5+400μLDilution	2
NCS（Negative control）	Dilution	0

Assay Procedure

(I) Sample loading and incubation

1. Add detection antibody: Take 1× Pichia pastoris HCP enzyme-labeled antibody solution into the loading groove, and use a multichannel pipette to quickly add 100 μL/well of the antibody solution to the bottom of the microplate well without introducing bubbles. In actual detection, samples can be added according to the number of samples (refer to Table 4 for example for 96-well plate layout).
2. Add calibrators and samples to be tested: Accurately pipette 100 μL of series calibrators, diluent (0 value),

and samples to be tested into the corresponding microplate. It is recommended to make 2-3 parallel wells for each concentration, and record the location of each concentration well.

3. After the sample is added, seal the microplate with a sealing film, place it on a microplate constant temperature oscillator, and incubate at room temperature at 600 rpm in the dark for 3 hours.

	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						

This example shows the detection of 6 concentration gradient calibration curves (ST1-ST6), 1 negative control (NCS), 3 samples to be tested (S1-S3) and the spike recovery of each sample (S1+SRC-S3+SRC).

In actual testing, you can refer to this example to perform 96-well plate layout and sample addition according to the number of samples.

Customers can determine the number of daily test replicates and whether to set spike recovery based on the method validation results.

(II) Color development

1. Balance the TMB colorimetric solution at room temperature 20 minutes in advance.
2. Wash the above plate with 1× buffer, 300 µL/well, quickly shake off the liquid, pat dry on a paper towel, and repeat the plate washing 5 times. The microplate should be immediately processed after washing and should not be placed.
3. Take a suitable volume of TMB colorimetric solution in the sample loading tank, use a multichannel pipette to quickly add 100 µL/well of TMB colorimetric solution to the above microplate, and incubate at room temperature in the dark for 10 min. Do not seal with a sealing film in this step.

(III) Termination

1. Take a suitable volume of stop solution in the sample loading tank, use a multichannel pipette to quickly add 50 µL/well of stop solution to the above microplate.
2. After termination, place the microplate at room temperature for 5 min.

(IV) Reading

1. Set the wavelength of the microplate reader to 450 nm and 620-650 nm (single wavelength within the range of 620-650 nm is acceptable), and measure the OD value of each well. Do not cover the sealing film or lid during measurement

Calculation

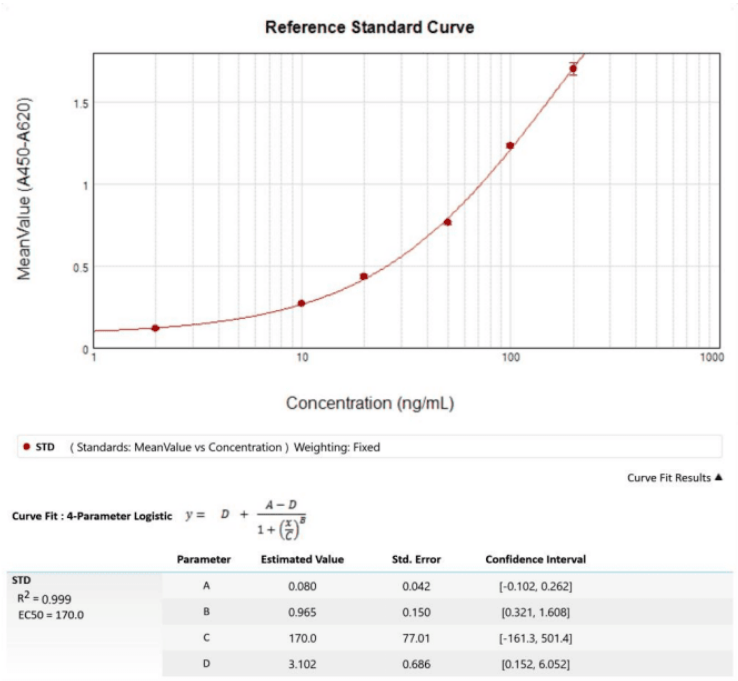
1. The OD450 nm value of each well needs to be subtracted from the long-wavelength OD value of each well. If the microplate reader is not equipped with a long wavelength, this step can be omitted.

2. After subtracting the OD value of the negative control (NCS) from the OD value of each calibration point and sample, the average value of the duplicate wells is taken.
3. Use the calibration point concentration value and the OD average value after processing in step (2) as the X-axis and Y-axis parameters for curve fitting (a total of 6 points) to obtain the calibration curve equation. It is recommended to use four-parameter fitting first. The calibration curve can be fitted using the software provided by the microplate reader. If it is not available, it is recommended to use professional calibration curve fitting software such as Curve Expert, ELISA Calc, etc.
4. Substitute the sample OD average value as the Y value into the equation obtained in step (3), and calculate the sample concentration by back-calculating the X value. If the sample is tested after dilution, the final sample concentration = the measured value after dilution × the dilution factor.

Interpretation Of Results

1. Calibration curve performance: Typical calibration curve parameters are described in (IV). Calibration curves may vary due to differences in experimenters and environmental changes, which is normal.
2. Sample suitability: Calculate the recovery rate based on the sample results at multiple dilution factors and the appropriate spiked samples at the corresponding dilution factors, and evaluate its dilution linearity, which should meet the methodological validation requirements of relevant regulations.
3. Sample report results: The same sample is tested at multiple dilution factors, and the sample results are the average of the multiple dilution factors that meet the sample suitability.

Typical Standard Curve



Detection Limit

LLOQ:2 ng/mL

Specificity

There is no obvious cross-reaction with host proteins of common exogenous expression system hosts, such as the prokaryotic expression host E. coli, the eukaryotic expression host CHO, the insect expression host Sf9, etc.; the cross-reaction rate with Saccharomyces cerevisiae and Hansenula officinalis is less than 1%.

Linearity

2-200 ng/mL, $R^2 \geq 0.990$

Precautions

The personnel who use the kit must be trained and qualified before use. In order to obtain satisfactory test results, please pay attention to the following points in advance:

All reagents must be prepared with sterile disposable pipettes, test tubes and sample slots, etc., and do not mix them. Avoid contamination of the connection part of the micropipette tip. It is recommended to wipe it with 75% alcohol before and after each experiment. Standardize the pipetting operation. It is strictly forbidden to suck the liquid into the pipette or place it horizontally on the table without removing the tip.

The dilution and mixing of the calibrator and the sample should be gentle and sufficient, and do not produce a lot of foam.

The stop solution is an acidic solution. Pay attention to the protection of the eyes, face, hands and clothes during use.

It is not recommended to mix different batches of kits.

The water used to prepare the buffer solution must be sterile water or freshly prepared ultrapure water, and the water temperature must not exceed 37 °C.

When adding the sample, add the sample to the bottom of the ELISA plate and try not to touch the wall of the hole. Be careful not to have bubbles, and you can shake it gently to mix. If there are bubbles before testing on the machine, they need to be punctured with a clean 10 µL pipette or needle. Be careful not to suck away the liquid in the well, which will cause large errors in the results.

During the incubation reaction, the ELISA plate needs to be covered with a film to prevent sample evaporation.

After pouring off the buffer, the subsequent solution should be added immediately. Do not let the ELISA wells dry to prevent affecting the detection performance of the kit.

Unused ELISA strips need to be stored in the self-sealing aluminum foil bag attached to the kit to avoid contamination by other samples, which will cause the kit to be scrapped.

The preparation of calibrators and sample dilutions must be accurate. The minimum sampling volume during preparation should not be less than 5 µL to prevent large errors in the results.

Pichia pastoris HCP enzyme-labeled antibody (100×) Please quickly centrifuge before use and shake the remaining reagent in the tube cap to the bottom of the tube to prevent contamination and loss of the reagent.

Calibrators and 1× Pichia pastoris HCP enzyme-labeled antibodies that have been diluted to the working concentration are not recommended for reuse because their stability cannot be verified.

TMB solution should be a colorless and transparent liquid. When aspirating, be sure to replace the clean tip to prevent HRP contamination. If a light blue color is found, please discard it.

Make sure to test on the machine 5-10 minutes after adding the stop solution, the result will be more stable, and the time should not exceed 30 minutes.

Because sodium azide can inhibit HRP activity and has a great impact on the test results, sodium azide cannot be added to the sample.

Limitations

1. This product is only for research purposes and not for clinical diagnosis.
2. Too low or too high pH value of sample solution may exceed the buffer range of diluent, thus affecting the performance of the kit. It is recommended to control the pH of sample solution between 6.5-8.5.
3. The components of sample matrix and preparation should not be products expressed by Pichia pastoris, otherwise the sample quantification may be abnormal due to the residue of Pichia pastoris HCP in the components.
4. If the main product in the sample has protease activity, the sample quantification may be abnormal due to enzyme degradation. It is recommended to inactivate the main product by appropriate means before testing.