Instructions for Use of E.coli HCP ELISA Detection Kit (2G)

The kit is intended for scientific research only and should not be used for diagnosis

Cat. No. HG-HCP002-2G

Product Introduction

This product uses a double antibody sandwich method to detect E.coli host cell protein (HCP) residues in samples. Using a microtiter plate pre-coated with E.coli HCP capture antibody, after adding the sample and standard, the E.coli HCP capture antibody specifically captures E.coli HCP, washing away impurities and supernatant, then adding enzyme-labeled antibody, washing away unbound enzyme-labeled antibody, adding chromogenic substrate for chromogenic reaction, and reading the absorbance after stopping the reaction. The absorbance value of the sample is positively correlated with the amount of E.coli HCP detected in the sample. Comparing it with the standard curve and multiplying by the corresponding dilution factor gives the residual amount of E.coli HCP in the sample.

Detection range: 1.5625 - 100 ng/mL

Limit of quantitation: 1 ng/mL

Limit of detection: 0.3 ng/mL

Precision: CV% ⩽ 10%, RE% ⩽ ±15%

Specification

96T

Usage

This product can be used for the quantitative detection of residual E.coli HCP in various biological products and pharmaceuticals, including intermediates, semi-finished products, and final products. This product has been tested and is suitable for most E.coli engineered cells, such as DH5α and BL21, etc. This detection kit is for scientific research and industrial use only and should not be used for any clinical diagnosis.

Kit components

Component	Strength	Preparation	Storage temperature
Standard	1 mL × 8 tubes (ST1-ST7, NTC)	Ready-to-use	-20°C
100 × Enzyme-labeled antibody (100 ×Anti-E.coli HCP-HRP)	200 µL × 1 tube	Dilute 1:99 with sample diluent	
Sample Diluent	15 mL × 2 bottles	Ready-to-use	Store unopened at -20°C, after opening store at 2-8°C
20 ×Wash Buffer	25 mL × 2 bottles	Dilute 1:19 with sterile water for injection	
Stop Solution	10 mL × 1 bottle	Ready-to-use	

Component	Strength	Preparation	Storage temperature
TMB Substrate A	6 mL × 1 bottle	Ready-to-use	Store unopened at -20°C, after opening store at 2-8°C
TMB Substrate B	6 mL × 1 bottle	Ready-to-use	
Coated Plate	8 wells × 12 strips	Ready-to-use	
Sealer film	5 pieces	Ready-to-use	
Instruction For Use	1 copy	Ready-to-use	

Storage conditions and expiry date

Unopened kits are valid for 12 months, stored at specified temperature.

Materials to be self-prepared

- Microplate reader
- Centrifuge
- Micropipettes and tips
- Thermostatic incubator
- Sterile water for injection
- Vortex oscillator
- Absorbent paper

Reagent preparation

1. Equilibration: Bring the required reagents to room temperature (18-25°C) and equilibrate for 30 minutes.
2. Preparation:

Preparation of 1×washing buffer: Equilibrate 20×washing buffer to room temperature, without crystallization. After mixing well, according to the amount used, dilute an appropriate amount of 20x washing buffer by 20 times with sterile water for injection at the ratio of 1:19, to obtain 1x washing buffer;

Note: It is normal that if a small amount of crystallization appears at the mouth or inside the 20x washing buffer bottle. Heat at 37°C using a metal bath or water bath for a period of time to dissolve the crystals.

3. Standard Dilution: According to experimental volume, dilute the standards A1-A7 with sample diluent at 1:1.

Dilution tube	Dilution volume	Concentration
A1	150 µL ST1 + 150 µL sample diluent	100ng/mL
A2	150 µL ST2 + 150 µL sample diluent	50ng/mL
A3	150 µL ST3 + 150 µL sample diluent	25ng/mL
A4	150 µL ST4 + 150 µL sample diluent	12.5ng/mL
A5	150 µL ST5 + 150 µL sample diluent	6.25ng/mL
A6	150 µL ST6 + 150 µL sample diluent	3.125ng/mL
A7	150 µL ST7 + 150 µL sample diluent	1.5625ng/mL
Negative (A8)	150 µL NTC + 150 µL sample diluent	0ng/mL

Operation procedures

All reagent components and samples to be tested should be restored to room temperature (18-25°C) before use. Duplicate well assay is recommended for all standards and samples to be tested.

1. Plate washing: Calculate the microtiter strips required for the samples to be tested and standards, remove the microtiter strips from the aluminum foil bag, put the remaining microtiter strips back into the aluminum foil bag and seal the bag, and store it at 2-8°C. Once the microtiter strips return to room temperature (18-25°C), add 300 µL/well of the prepared 1x washing buffer to each well of the microtiter plate, let stand for 30 seconds, discard all liquid, and thoroughly pat dry on clean absorbent paper, repeat the washing process 3 times.

Note: When discarding the liquid, avoid backflow or splashing that could contaminate other wells. When patting dry, change the paper timely and do not repeatedly pat the microtiter plate at the same spot. Ensure the absorbent paper is clean and dry before each patting.

2. Preparation of sample solution: Based on the estimated sample content, take an appropriate amount of sample and dilute adequately with sample diluent.

3. Preparation of spike-in control solution (ERC): Take 150 µL of ST3 standard (50 ng/mL), add 150 µL of the diluted sample, and mix well.

4. Loading: Sequentially pipette 100 µL/well of each standard from A8, A7 (1.5625 ng/mL), A6 (3.125 ng/mL), A5 (6.25 ng/mL), A4 (12.5 ng/mL), A3 (25 ng/mL), A2 (50 ng/mL), A1 (100 ng/mL), sample solutions, and spike-in controls into the corresponding wells of the microtiter plate, each set up replicate well.

5. Sample incubation:After adding samples, cover the microtiter plate with sealer film and incubate the plate at 37°C for 1 hour.

6. Plate washing: After incubation, remove the microtiter plate, let it stand at room temperature (18-25°C) for 3-5 minutes, remove the sealer film, discard the liquid, add 300 µL/well of 1x washing buffer to each well of the microtiter plate, let stand for 30 seconds, discard all liquid, and thoroughly pat dry on clean absorbent paper, repeat the washing process 3 times.

7. Preparation of enzyme-labeled antibody: About 2 minutes before the completion of sample incubation, take out the enzyme-labeled antibody. According to the experimental volume, dilute the enzyme-labeled antibody 100 times with sample diluent at 1:99, and newly prepared and ready-to-use.

8. Adding enzyme-labeled antibody: Add 100 µL/well of the diluted enzyme-labeled antibody into the corresponding wells.

9. Incubation of enzyme-labeled antibodies: After adding the enzyme-labeled antibody, cover the microtiter plate with sealer film and incubate the plate at 37°C for 1 hour. Upon completion of incubation, take out TMB Substrate A/B from the 4°C refrigerator and let it stand at room temperature (18-25°C) for standby.

10. Plate washing: After incubation, remove the microtiter plate, let it stand at room temperature (18-25°C) for 3-5 minutes, remove the sealer film, and discard the liquid. Add 300 µL/well of 1x washing buffer to each well of the microtiter plate, let stand for 30 seconds, discard all liquid, and thoroughly pat dry on clean absorbent paper, repeat the washing

process 6 times.

11. Chromogenic reaction: According to the experimental volume, take the required amount of TMB Substrate A/B and mix in a 1:1 ratio, newly prepared for immediate use. Using a multichannel pipette and a clean reagent reservoir, add 100 µL/well of the TMB Substrate A/B mixture, incubate at room temperature (18-25°C) in the dark for 30 minutes (do not shake during color development).

12. Termination:After the chromogenic reaction is complete, use a multichannel pipette and a clean reagent reservoir to add 50 µL/well of stop solution.

13. Readings:Read the absorbance value at 450 nm/650 nm within 20 minutes. Take 450 nm as detection wavelength and 650 nm as reference wavelength.

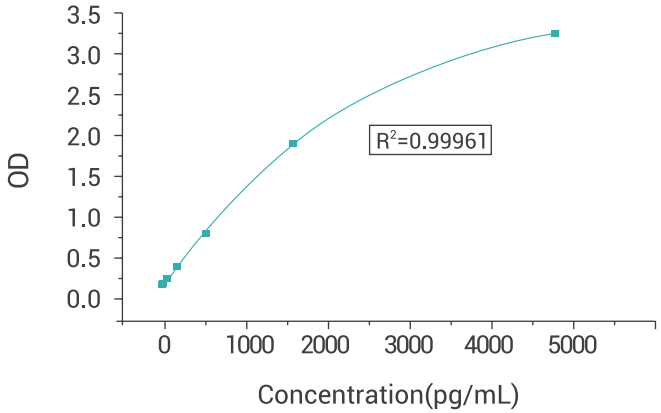
14. Data processing: Use the absorbance of the standards as the y-axis and concentration as the x-axis to fit the response signal difference between OD450-OD650 nm using a four-parameter equation. Substitute the sample OD values into the equation to get the detected value of HCP residue, then multiply by the dilution factor to obtain the sample host cell protein residue concentration.

Result processing

1. OD processing of the standard curve (See the following example. For example only, please refer to the actual measurement for details)

Concentration of the standards (ng/mL)	OD1	OD2	Mean value	CV(%)	Test value (ng/mL)	Recovery (%)
100	1.38430	1.41080	1.39755	2	100.06497	100
50	0.90180	0.91590	0.90885	2	49.87385	100
25	0.54860	0.55260	0.55060	1	25.09237	100
12.5	0.32590	0.32790	0.32690	1	12.64237	101
6.25	0.19510	0.19310	0.19410	1	6.00335	96
3.125	0.13490	0.13270	0.13380	2	3.11057	100
1.5625	0.10600	0.09870	0.10235	6	1.61054	103
0	0.0694	0.07	0.0697	4	0	/

(2) A standard curve is obtained by fitting the theoretical concentration of the standard and the corresponding OD value with four parameters (shown in the figure below).



Precautions

All reagent components and samples to be tested should be restored to room temperature (18-25°C) before use. Duplicate well assay is recommended for all standards and samples to be tested.

1. For the first detection of samples, it is recommended to perform at least three consecutive dilutions to produce at least one diluted sample within the range of the standard curve.
2. Reagents shall be stored according to label instructions and equilibrated at room temperature (18-25°C) before use.
3. Before using the coated plate, please let it equilibrate to room temperature (18-25°C) and then open the secondary package. The strips not used in the experiment shall be immediately placed back in the package for sealing and can be stored at 4°C for one month. The remaining reagents shall be packaged or covered.
4. Please use disposable tips during experiments to avoid cross-contamination.
5. Check various reagents in the kit before use. Dilution, spiking and termination of the reaction with reagents shall be thoroughly mixed or shaken well, which is particularly important for the experimental results.
6. The residual washing buffer in the reaction wells during the washing process shall be patted thoroughly on a clean tissue until no watermark is visible. Do not place the tissues into the reaction wells to absorb water.
7. The substrate chromogenic reagent is sensitive to light, avoid prolonged exposure to light and avoid contact with metals that may affect the results.
8. This product is a disposable kit and shall be used within the validity period.

Frequently asked questions and solutions

Problems	Possible Causes	Solutions
Positive result in negative control	Contamination of samples or reagents, or cross-contamination due to spilling of solution between adjacent wells as a result of improper handling when adding samples	Replace reagents and operate cautiously.
	Incomplete washing of the microtiter plate	Before washing the plate, discard the antibody solution completely, then fill the wells with washing buffer to ensure adequate washing.

Problems	Possible Causes	Solutions
High overall background on the microtiter plate	Too long reaction time	Immediately use stop solution to terminate the reaction once the chromogenic reaction is sufficient for absorbance reading, and appropriately shorten the color development time.
	Substrate incubation was not protected from light.	Substrate incubation should be protected from light
Standard curve does not develop color or develops very weak color, while samples develop color	Inadequate or no vortexing during serial dilution of standards.	Use vortexing during dissolution and dilution mixing.
No color development in both the standard curve and samples.	Omission of a component, especially detection antibody or enzyme.	Check test records and remaining reagents. Verify labels before each addition of liquid.
	Expired reagents	Use products within the expiry date.
	Inactivation or loss of standards/antibody/ enzyme/chromogenic substrate;	Correctly store and replace with new standards/antibody/enzyme/ chromogenic substrate.
Both the standard curve and samples develop color, but the CV of the replicate wells is high	Pipettors and tips are not compatible	Replace pipette tips.
	Poor pipette airtightness	Use and maintain pipettes correctly and according to standard procedures
	Inconsistent pipetting technique	Practice pipetting repeatedly to maintain consistency.
Large differences in calculated sample values after different gradient dilutions.	Strong matrix effect of the sample	Select the dilution factor where the calculated sample values are close under two specific dilution gradients (for samples with higher levels of the target protein)

Disclaimer

Under all circumstances, the liability of our company for this product is only limited to the value of the product itself.

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