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

Product Summery

This Kit employs a double-antibody sandwich ELISA to detect residual E. coli host cell proteins (HCP) in samples. A microplate pre-coated with E. coli HCP capture antibody is used. After adding samples and standards, the E. coli HCP is specifically captured by the coated antibody. Unbound substances are removed by washing, followed by the addition of an enzyme-linked detection antibody. After another wash to remove unbound detection antibody, a chromogenic substrate is added for color development. The reaction is stopped, and the absorbance value is measured. The absorbance value correlates positively with the amount of E. coli HCP in the sample. By comparing the sample absorbance to a standard curve and multiplying by the corresponding dilution factor, the residual E. coli HCP concentration in the sample can be determined.

Measuring Range: 3.125 - 200 ng/mL
Quantitative Limit： 1 ng/mL
Lower Limit of Quantification： 0.3 ng/mL
Precision： CV% ≤ 10%, RE% ≤ ±15%

Cat. No. & Specifications

Cat. No.	Specification	Shelf Life
HG-HCP002-2G	96T	12 Months
HG-HCP002-2G-S	48T	

Applications

This kit is designed for the quantitative detection of residual E. coli host cell proteins (HCP) in intermediates, semi-finished, and finished products of biologics and pharmaceuticals. Validated for compatibility with common E. coli expression strains (e.g., DH5α, BL21, TOP10). For research and industrial use only. Not for clinical diagnosis.

Kit Component

Component Name	Specification	Used Way	Storage Temperature
E.coli HCP Standard	1 mL×8 tubes (ST1-ST7，NTC)	Pre-dilution	-20℃
100×Anti-E.coli HCP-HRP	200 μL×1 tube	1:99 dilute with Sample Diluent	
Sample Diluent	15 mL×2 bottles	Pre-dilution	Store at -20℃ before unpacking; Store at 2-8℃ after opening.
20×Wash Buffer	25 mL×2 bottles	1：19 dilute with sterile water	
Stop Solution	10 mL×1 bottle	Pre-dilution	

TMB Substrate A	6 mL×1 bottle	Pre-dilution	
TMB Substrate B	6 mL×1 bottle	Pre-dilution	
Coated Plate	8 wells x 12 strips	Pre-dilution	
Sealer film	5 films	Pre-dilution	

Storage & Stability

Unopened kit: 12-month shelf life when stored at specified temperatures.

Materials Required (Not Included):

- Microplate reader
- Centrifuge
- Micropipettes & tips
- Incubator
- Sterile water for injection
- Vortex mixer
- Absorbent paper

Reagent Preparation

1. Equilibration: Allow all reagents to reach room temperature (18–25°C) for 30 min.

2. Preparation of Wash Buffer (1×):

Bring the 20× Wash Buffer to room temperature and ensure it is free of any crystals. Mix thoroughly, then dilute with sterile water for injection at a ratio of 1:19 according to usage requirements to obtain the final 1× Wash Buffer.

Note: The appearance of a small amount of crystallization at the bottle mouth or inside the 20× Wash Buffer is a normal phenomenon. It can be resolved by heating the buffer at 37°C using a metal bath or water bath for a period of time until the crystals dissolve.

Procedure

All reagents and samples must equilibrate to 18–25°C. Duplicate wells recommended.

1. Sample Preparation: Dilute samples with Sample Diluent (optimize dilution factor).

2. Extraction Recovery Control (ERC) Preparation: Mix 150 µL ST3 (50 ng/mL) + 150 µL diluted sample.。

3. Sample Loading: Pipette 100 µL per well of the following solutions into the plate wells in sequence: NTC, ST7 (3.125 ng/mL), ST6 (6.25 ng/mL), ST5 (12.5 ng/mL), ST4 (25 ng/mL), ST3 (50 ng/mL), ST2 (100 ng/mL), ST1 (200 ng/mL), sample solutions, and ERC. All samples are added in 2 duplicates.

4. Sample Incubation: After sample loading, seal the microplate with plate sealing film and incubate at 37°C for 1 hour.

5. Plate Washing: After incubation, remove the plate and let it stand at room temperature (18–25°C) for 3–5 minutes. Remove the sealing film and discard the liquid by inverting the plate. Add 300 µL of 1× Wash Buffer to each well, let it stand for 30 seconds, discard the liquid, and blot dry thoroughly on clean absorbent paper. Repeat the wash process 3 times.
Note: Avoid backflow or splashing to prevent cross-contamination between wells. When blotting, change the absorbent paper frequently. Do not pat the plate on the same spot of the paper repeatedly, and ensure the absorbent paper is clean and dry before each use.

6. HRP-Conjugated Antibody Preparation: Approximately 2 minutes before sample incubation ends, take out the HRP-conjugated antibody. Dilute 100× Anti-E.coli HCP-HRP with Sample Dilution Buffer at a 1:99 ratio to prepare the working solution. Prepare fresh before use.

7. Antibody Addition: Add 100 µL of the prepared HRP-conjugated antibody solution to each designated well.

8. Antibody Incubation: Seal the plate with sealing film again and incubate at 37°C for 1 hour. Near the end of incubation, take Substrate Solution A and B out of the 4°C refrigerator and bring them to room temperature (18–25°C) for later use.

9. Plate Washing 2: After incubation, let the plate stand at room temperature (18–25°C) for 3–5 minutes. Remove the sealing film and discard the liquid. Add 300 µL of 1× Wash Buffer to each well, let it stand for 30 seconds, discard the liquid, and blot dry thoroughly on clean absorbent paper. Repeat the wash process 6 times.

10. Color Development: Prepare the required volume of TMB Substrate A and B freshly by mixing them at a 1:1 ratio. Using a multichannel pipette and a clean reagent trough, add 100 µL of the TMB substrate mixture to each well. Incubate at room temperature (18–25°C) in the dark for 30 minutes. Do not shake the plate during color development.

11. Stop Reaction: After color development, use a multichannel pipette and a clean reagent trough to add 50 µL of Stop Solution to each well.

12. Data Reading: Read the absorbance at OD_{450} nm (detection wavelength) and 630 nm (reference wavelength) within 20 minutes.

13. Data Analysis: Plot the absorbance (OD_{450}nm–OD_{630} nm) of the standards on the Y-axis and their concentrations on the X-axis to perform a 4-parameter logistic (4PL) curve fitting. Substitute the OD values of the samples into the equation to calculate the residual host cell protein content, then multiply by the dilution factor to obtain the final concentration of host cell protein in the sample.

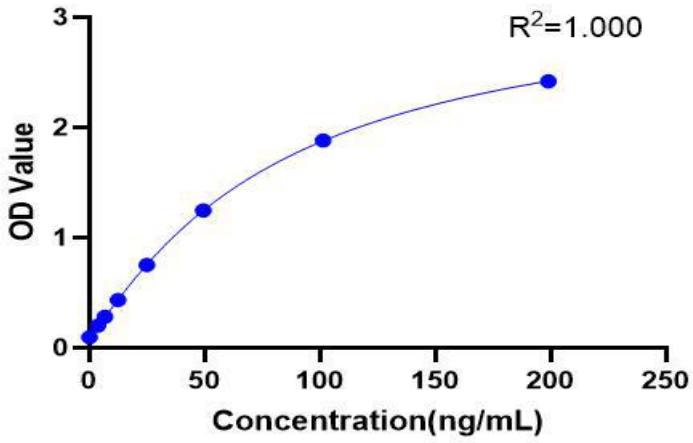
Process Flowchart

Data Process and Result

OD Standard curve processing (see the example below, only for example, the specific details are subject to actual measurement)

Standard Concentration(ng/mL)	OD1	OD2	Average	CV	Measured Value (ng/mL)	Recovery Rate (%)
200	2.39410	2.44480	2.41945	1%	199.00153	100
100	1.88370	1.87950	1.88160	0%	101.28970	101
50	1.23470	1.25560	1.24515	1%	49.43616	99
25	0.74080	0.76200	0.75140	2%	24.96131	100
12.5	0.42850	0.43670	0.43260	1%	12.37254	99
6.25	0.28410	0.27730	0.28070	2%	6.71947	108
3.125	0.20400	0.19980	0.20190	1%	3.71466	119
0	0.09720	0.09500	0.09610	2%	0	/

1. A four-parametric nonlinear regression model was applied to plot the standard curve, correlating the nominal standard concentrations with their measured optical densities (OD)."



Precautions

All reagents and test samples should be brought to room temperature (18–25°C) before use. It is recommended to perform all standard and sample tests in 2 duplicates.

1. For initial sample testing, it is recommended to prepare at least 3 serial dilutions to ensure at least one diluted sample falls within the standard curve range.
2. Store reagents according to the label instructions. Allow reagents to equilibrate to room temperature (18–25°C) before use.
3. Bring the coated ELISA plate to room temperature (18–25°C) before opening the foil pouch. Unused wells should be immediately returned to the pouch, sealed, and stored at 4°C, where they remain stable for one month. Unused reagents should also be properly sealed or covered.
4. Use disposable pipette tips throughout the assay to avoid cross-contamination.
5. Check all reagents before use. Proper mixing or shaking during reagent dilution, sample addition, and reaction termination is crucial for accurate results.
6. During washing, any residual liquid in the wells should be thoroughly blotted on clean absorbent paper until no visible moisture remains. Do not insert paper directly into the wells.
7. The substrate solution is light-sensitive. Avoid prolonged exposure to light and contact with metal surfaces, which may affect the results.
8. This product is for single-use only. Use within the indicated expiration date.

Common Problems and Solutions

Problems	Possible causes	Workaround
NTC showed positive result	The samples and reagents are contaminated, or improper operation during sample addition leads to splashing of solutions between adjacent wells, resulting in cross contamination.	Replace reagents.
	The ELISA plate was not washed thoroughly.	Before washing the plate, pour out the antibody solution and then fill the wells with the washing solution to ensure adequate washing.
The plate exhibits elevated background signal across all well	Over reaction time	Terminate the reaction immediately with stop solution once sufficient color development is achieved for optical density (OD) measurement. Appropriately reduce the incubation time to prevent over-development.
	Do not protect from light during the incubation	Protect from light during the incubation
The standard curve does not show color or the color is very weak but the samples show color	When the standard was diluted in multiples, the vortexing was not sufficient or insufficient.	Vortex the sample thoroughly during solution preparation and dilution to ensure homogeneous mixing.
The standard curve does not show color and also the samples do not show color	Missing Reagent Components, the detection antibody or enzyme conjugate was accidentally omitted during the assay procedure.	Review experimental records and remaining reagents. Verify labels prior to each liquid addition.
	Expired reagents	Use the reagent during the shelf life.
	The standard curve was invalid due to degraded standards, inactivated detection antibody, or unstable enzyme/substrate.	Store the ST/antibody/enzyme/substrate with correct conditions.
Both the standard curve and sample show color, but the CV is so high.	The pipette and the tip do not match.	Replace the tip.
	Poor air tightness of pipette.	Correct and standardized use and maintenance of pipettes.
	Inconsistent pipetting techniques.	Practice pipetting repeatedly to maintain consistency
Due to pronounced matrix effects, the derived sample concentrations varied substantially across serial dilutions.	The sample have a strong matrix interference.	For samples with high target protein content, select two dilution gradients where the calculated values converge.

Disclaimer

In all circumstances, our company's liability for this product shall be limited solely to the value of the product itself.

江苏谱新生物医药有限公司
A. 苏州市吴中区吴中大道 1463 号越旺智慧谷 4 号楼
T.400 900 1882 W.www.hillgene.com