TECHNICAL MANUAL

E.coli HCP PharmaGenie ELISA Kit

- **SKU CODE:** AEGE00001
- **SIZE:** 96T
- **DETECTION PRINCIPLE:** Sandwich
- **RUO:** Research-Use-Only

Revised: 08/2025

E.coli HCP PharmaGenie ELISA Kit

Please read entire manual carefully before starting experiment.

Table of Contents

1. Key Features

Assay Range:

1.5625 - 100 ng/mL

Limit of quantification:

1 ng/mL

Limit of detection:

0.3 ng/mL

Detection Method:

Sandwich

Sample Type:

E.coli engineered cells, such as DH5α and BL21, etc

Precision:

CV% ≤ 10%, RE% ≤ ±15%

2. Storage & Expiry

Assay Genie ELISA Kits are shipped on ice packs. Please store this ELISA Kit as indicated in section 4. Validity for 12 months . Date of expiration is on the ELISA Box label.

3. Product Description

The Assay Genie E.coli HCP PharmaGenie ELISA Kit utilizes a highly specific double-antibody sandwich method to accurately detect residual E. coli host cell proteins (HCP) in samples. This kit is designed for use in biopharmaceutical process development and quality control, where monitoring and quantifying residual HCPs is essential to ensure product purity, safety, and regulatory compliance. Typical applications include the analysis of in-process samples, purification intermediates, and final drug substance to verify the effective removal of HCP contaminants during upstream and downstream processing.

Each microtiter plate is pre-coated with an E. coli HCP capture antibody to ensure reliable binding of target proteins. After the addition of samples and standards, E. coli HCP present in the sample is specifically captured, while unbound components are removed through washing.

An enzyme-conjugated detection antibody is then applied to form an antibody-antigen-enzyme complex, followed by additional washing steps to remove any unbound conjugate. A chromogenic substrate is subsequently added to initiate color development, and the reaction is terminated to allow absorbance measurement. The absorbance value is directly proportional to the concentration of E. coli HCP in the sample. Residual HCP levels are determined by comparison to the standard curve and adjusted for the sample's dilution factor.

4. Kit Contents

No	Component Name	Size	Preparation	Storage
1	Standard	1 mL x 8 tubes (ST1-ST7, NTC)	Ready-to-use	-20°C
2	100 x Enzyme-labelled antibody - 20℃ (100 x Anti-E.coli HCP-HRP	200 µL × 1 tube	Dilute 1:99 with sample diluent	
3	Sample Diluent	15 mL × 2 bottles	Ready-to-use	Store unopened at -20°C, after opening store at 2-8 °C
4	20 x Wash Buffer	25 mL × 2 bottles	Dilute 1:19 with sterile water for injection	
5	Stop Solution	10 mL × 1 bottle	Ready-to-use	
6	TMB Substrate A	6 mL × 1 bottle	Ready-to-use	
7	TMB Substrate B	6 mL × 1 bottle	Ready-to-use	
8	Coated Plate	8 wells × 12 strips	Ready-to-use	
9	Sealer film	5 pieces	Ready-to-use	
10	Technical Manual	1 copy		-

Additional materials required:

1. 37°C incubator.
2. Plate Reader with 450nm filter.
3. Precision pipettes and disposable pipette tips.
4. Distilled water.
5. Disposable tubes for sample dilution.
6. Absorbent paper.

5. Precautions

1. Store all reagents according to the instructions on the product label. Before use, allow all reagents to equilibrate to room temperature.
2. Before opening the secondary packaging, bring the pre-coated strip plates to room temperature. Return any unused strips immediately to the original packaging and reseal tightly. Store unused plates at 4°C for up to one month. All other unused reagents should be properly sealed or covered.
3. The volumes of the standard, biotinylated antibody, and enzyme conjugate are small. Perform a quick centrifugation prior to use to ensure that any liquid adhering to the tube walls or caps collects at the bottom.
4. Always use disposable pipette tips during the assay to prevent cross-contamination.
5. Inspect all kit components before use. To ensure accurate results, mix thoroughly when preparing dilutions, loading samples, or adding stop solution.
6. During the washing steps, after removing Wash Buffer, tap the plate dry on clean absorbent paper until no residual droplets or watermarks are visible. Do not insert tissue directly into the wells.
7. The TMB substrate is photosensitive, protect it from prolonged light exposure. Avoid contact with metal surfaces, as this may interfere with the reaction.
8. This kit is intended for single use and should be used within its stated shelf life.

6. Sample Preparation

Due to the inherent variability of biological samples and the specific requirements of individual assays, users are advised to optimize protocols in accordance with their own experimental conditions. Samples may be tested directly with this ELISA or diluted as necessary, based on experimental objectives and the physicochemical characteristics of the sample matrix.

***Note**: For information regarding validation data in specific samples, please contact our Technical Support Team at techsupport@assaygenie.com.*

7. Reagent Preparation

1. **Equilibrate reagents:** Bring the required reagents to room temperature (18-25°C) and equilibrate for 30 minutes.
2. **Wash Buffer Preparation:** Equilibrate 20x 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℃ 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 or using 7 Eppendorf Tubes with sample diluent at 1:1.

No	Dilution Volume	Final concentration (µg/mL)
1	150 µL ST1 + 150 µL sample diluent	100ng/mL
2	150 µL ST2 + 150 µL sample diluent	50ng/mL
3	150 µL ST3 + 150 µL sample diluent	25ng/mL
4	150 µL ST4 + 150 µL sample diluent	12.5ng/mL
5	150 µL ST5 + 150 µL sample diluent	6.25ng/mL
6	150 µL ST6 + 150 µL sample diluent	3.125ng/mL
7	150 µL ST7 + 150 µL sample diluent	1.5625ng/mL
8	150 µL NTC + 150 µL sample diluent	0ng/mL

8. Assay Procedure

Note: *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 (1.562ng/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. **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. **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 colour 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.

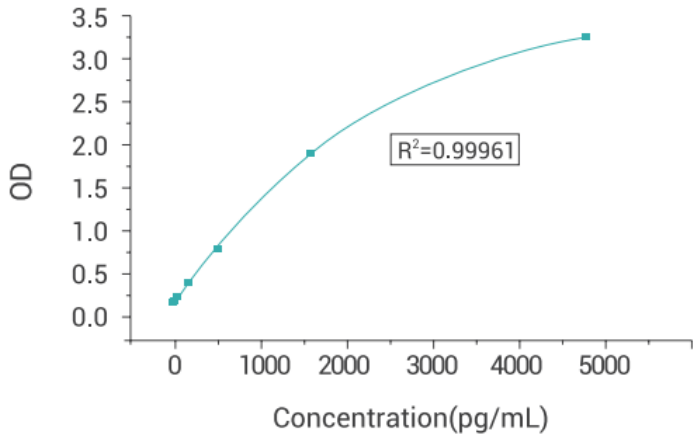
9. Data Analysis

The 4-parameter fitting method is recommended for the linear fitting and calculation of the product.

OD processing of the standard curve (the following example is provided as reference only, and the results from actual detection shall prevail).

Std concentration (ng/mL)	OD Value (1)	OD Value (2)	Mean value
100	1.38430	1.41080	1.39755
50	0.90180	0.91590	0.90885
25	0.54860	0.55260	0.55060
12.5	0.32590	0.32790	0.32690
6.25	0.19510	0.19310	0.32690
3.125	0.13490	0.13270	0.13380
1.5625	0.10600	0.09870	0.10235
0	0.0694	0.09870	0.0697

The standard curve is obtained by 4-parameter fitting with the theoretical standard concentrations and the corresponding OD values (as shown in the figure below).



10. ELISA Troubleshooting

Problem	Possible Causes	Solutions
Standard curve without signal	Incorrect reagent order; Mixed components from different kits; Missing reagents.	Ensure correct reagent order and use components from the same kit. Verify all reagents are added.
Overflow OD	Mixed components from different kits; Over-concentrated working solution	Use correct components and prepare solutions at recommended concentrations.
Poor standard curve	Incorrect curve fitting model.	Try alternative curve fitting models.
Samples without signal	Sample concentration too low; Incompatible buffer; Incorrect preparation; Sample degradation or excessive freeze-thaw.	Reduce dilution or concentrate sample. Check buffer compatibility and follow proper preparation and storage.
High CV%	Precipitate formation; Unclean plate; Foaming; Uneven washing; Incomplete reagent mixing; Pipetting inconsistency.	Dilute samples if needed, avoid foaming, ensure uniform washing, mix reagents thoroughly, and use calibrated pipettes.
Low standard signal	Improperly reconstituted standards; Degraded standards; Incorrect pipetting; Expired kit; Improper storage; Over-dried wells.	Reconstitute standards properly, use fresh kits, follow storage recommendations, and prevent wells from drying.
Slow colour development	TMB not equilibrated; Incorrect microplate reader wavelength; Over-washing.	Pre-warm TMB (30 min at 37°C), confirm correct wavelength (450 nm), and follow recommended washing times.
High background	Insufficient washing; Contaminated wash buffer; Excess detection reagents; Delayed reading; TMB exposed to light.	Wash adequately, prepare fresh wash buffer, use correct reagent amounts, read results promptly, and incubate TMB in the dark.

Assay Genie 100% money-back guarantee!

If you are not satisfied with the quality of our products and our technical team cannot resolve your problem, we will give you 100% of your money back.