Instruction - E.coli HCP ELISA Kit

Cat. No.: ARK6626

For quantitative detection of E.coli Host Cell Protein (HCP) in biological products.

For research use only. Not for use in diagnostic procedures.

1. PRODUCT INFORMATION

1.1 Intended Use

This E.coli HCP ELISA Kit is a high-sensitivity kit for the detection of residual Host Cell Proteins (HCP) derived from E.coli BL21 strain in biological products. It can sensitively, specifically and accurately detect residual HCP of E.coli BL21 strain at different process stages of biological products.

1.2 Background

Escherichia coli (E.coli) is a common intestinal bacterium and a frequently used host cell line in biotechnology and biopharmaceutical industries. During the production of biological products using E.coli expression systems, a small amount of E.coli HCP may remain in semi-finished or finished products. Since E.coli HCP may have immunogenicity, biological activity or enzymatic activity, which can affect the activity of biological products, it is necessary to detect and control residual E.coli HCP during production. Therefore, detection of residual E.coli HCP is an important indicator for evaluating the production process of biological products.

1.3 Product Features

Short reaction time, stable results, high specificity, rapid detection and simple operation.

1.4 Principle

This kit adopts the double-antibody sandwich enzyme-linked immunosorbent assay for the determination of residual E.coli HCP in samples. Polyclonal antibody against E.coli HCP is pre-coated on the microplate. Standards and samples are added to the wells, followed by HRP-conjugated polyclonal antibody against E.coli HCP. E.coli HCP in the sample is captured by the immobilized antibody and binds to the enzyme-labeled antibody to form a “sandwich complex”. TMB substrate is then added for color development. The absorbance (OD value) is measured at 450 nm/630 nm using a microplate reader. The absorbance is positively correlated with the content of E.coli HCP in the sample.

1.5 Guidance for Method Validation

Laboratories using this kit are recommended to perform the following validations: Linearity, Range, Accuracy (Recovery), LOQ, LOD, Precision, Specificity, and Robustness. AREX can provide detailed quality control reports and performance validation reports. If users use the reports provided by AREX, only applicability validation for samples is required (including Precision, Recovery, Specificity, Robustness).

1.6 Performance Characteristics

- Limit of Detection (LOD): < 1 ng/mL
- Limit of Quantitation (LOQ): 3 ng/mL
- Linear Range: 1 – 243 ng/mL
- Accuracy (Recovery): 80% – 120%
- Accuracy (Bias): ≤ |±15%|
- Repeatability (Intra-assay CV): ≤ 10%

1.7 Specificity

Sample	Cross-reactivity	Sample	Cross-reactivity
E.coli HCP	100%	HEK293 HCP	< 0.01%
CHO HCP	< 0.01%	BSA	< 0.01%
Protein A	< 0.01%		

The validated sample types are limited. Each user is recommended to test cross-reactivity of known substances in their sample matrix in similar experiments.

1.8 Kit Components (96 Tests)

No.	Component	Size	Storage
1	Pre-coated Microplate	8×12 strips	2–8°C
2	Standard (2430 ng/mL)	500 µL × 1	2–8°C
3	Sample Diluent	30 mL × 1	2–8°C
4	HRP-Conjugated Antibody (10×)	1.5 mL × 1	2–8°C
5	Antibody Diluent	15 mL × 1	2–8°C
6	Wash Buffer (20×)	30 mL × 1	2–8°C
7	Chromogenic Solution A	8 mL × 1	2–8°C, dark
8	Chromogenic Solution B	8 mL × 1	2–8°C, dark
9	Stop Solution	15 mL × 1	2–8°C
10	Plate Sealer	3 pcs	–

1.9 Storage and Stability

The kit should be stored protected from light at 2–8°C, valid for 12 months. Unused kit after opening should still be stored protected from light at 2–8°C. See the kit label for production and expiration dates.

2. PROCEDURE

2.1 Preparation

2.1.1 Kit Equilibration

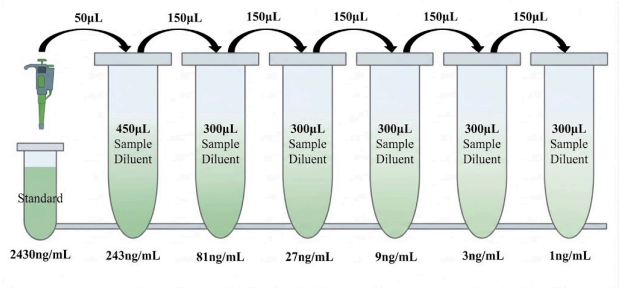
Bring all kit components to room temperature for 20 min before use.

2.1.2 Materials Required But Not Provided

1) Microplate reader, incubator/shaker, washer, vortex mixer, timer
2) Adjustable pipettes and tips
3) Deionized water
4) Absorbent paper, microtubes, gloves

2.1.3 Reagent Preparation

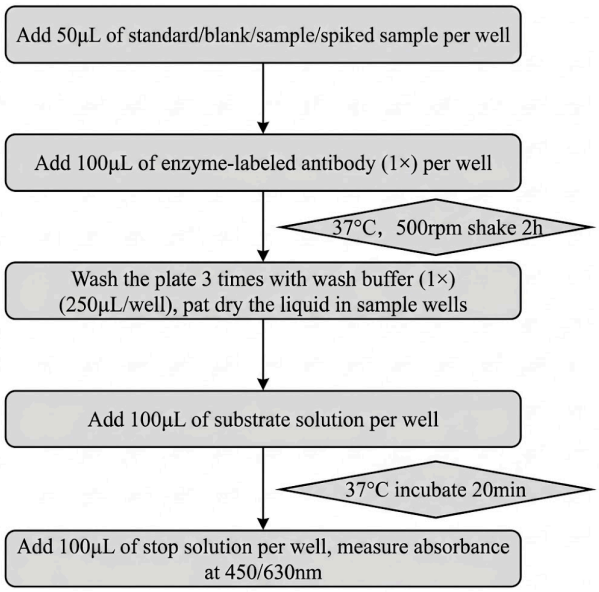
1) 1× Wash Buffer: Dilute 20× Wash Buffer 20-fold with deionized water. If crystals exist, dissolve at room temperature or 37°C before dilution.
2) 1× HRP-Conjugated Antibody: Dilute 10× antibody 10-fold with Antibody Diluent.
3) Working Chromogenic Solution: Mix equal volumes of Solution A and B immediately before use (within 10 min). Do not use if blue color appears.
4) Standard Preparation: Dilute 2430 ng/mL standard to 243 ng/mL with Sample Diluent, then perform 3-fold serial dilutions.



2.1.4 Sample Preparation

Bring samples to room temperature and mix well. For linearity validation, dilute samples into the linear range before testing.

2.2 Assay Protocol



1. Take required microplate strips. Mark the order. Seal unused strips and store at 2–8°C.
2. Add 50 μL standard/sample per well. Add 100 μL 1× HRP-conjugated antibody. Seal and incubate at 37°C with shaking at 500 rpm for 2 h.
3. Wash 3 times with 250 μL 1× Wash Buffer per well. Pat dry completely.
4. Add 100 μL working chromogenic solution per well. Incubate at 37°C in dark for 20 min.
5. Add 100 μL Stop Solution per well.
6. Read OD at 450/630 nm within 15 min.

3. DATA ANALYSIS

Calibrated Absorbance:

Calibrated OD = (OD_{450nm} − OD_{630nm}) – Blank OD

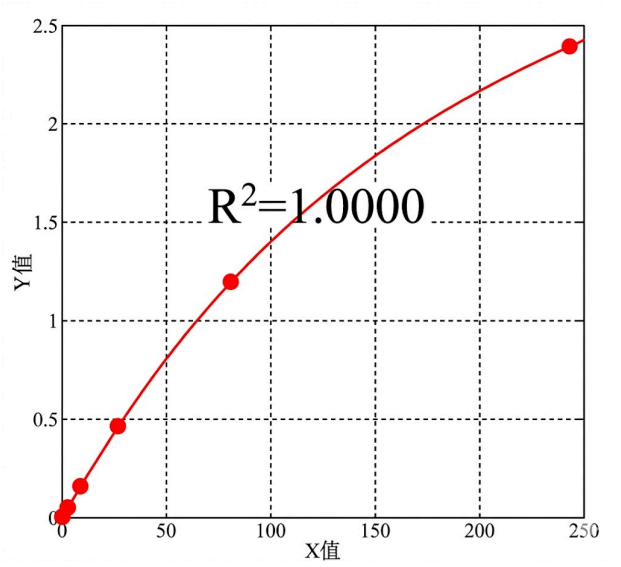
Standard Curve & Calculation:

Plot standard curve with concentration (X) vs calibrated OD (Y). Fit using 4-Parameter Logistic (4PL) model: Y = ((A − D) / (1 + (X/C)^B)) + D

Calculate concentration from calibrated OD, multiply by dilution factor to obtain actual HCP concentration.

Example Demonstration

Standard (ng/mL)	OD Value
243	2.3927
81	1.1983
27	0.4621
9	0.1576
3	0.0559
1	0.0212



4. PRECAUTIONS

1. All components must equilibrate to 20–25°C before use.
2. Mix all reagents well before use. Centrifuge standards briefly.
3. Always run a standard curve per assay. Do not mix reagents from different lots.
4. Avoid plate strip loss during drying steps.
5. Use only matched reagents; change tips to avoid cross-contamination.
6. Results depend on reagent quality, operator technique, and environment.
7. AREX is only responsible for the kit itself, not sample consumption.
8. For research use only; not for clinical diagnosis.

5. SAFETY INFORMATION

1. Stop Solution is acidic; handle with care.
2. All biological samples are potentially hazardous; handle and dispose according to regulations.
3. Wear PPE: lab coat, gloves, mask, goggles.

6. TROUBLESHOOTING

Problem	Possible Cause	Solution
Poor standard curve gradient	Incorrect dilution	Follow 3-fold dilution
	Pipetting error	Check pipettes and tips
	Incomplete washing	Ensure proper wash volume and times
Weak or no color	Short incubation	Use correct incubation time
	Incorrect temperature	Use recommended temperature
	Reagent volume error	Check reagent addition
	Wrong chromogen mix	Mix A+B fresh 10 min before use
Low OD reading	Wrong reader settings	Check wavelength; prewarm reader
High CV	Pipetting error	Improve technique
	Contaminated plate bottom	Clean plate bottom
	Bubbles/foreign matter	Ensure no bubbles
	Incomplete sealing	Seal plates fully
High background	Incomplete washing	Wash thoroughly; check washer
	Wrong incubation	Follow time/temperature
	Contaminated consumables	Use clean tubes and tips
	Contaminated buffer	Prepare fresh wash buffer

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