

# *E. coli* (K-12) HCP ELISA Kit

## Product Introduction

*Escherichia coli* (*E. coli*) K-12 strains and their derivatives, including DH5α, TOP10, and JM109, are widely used as host strains for plasmid DNA (pDNA) production in the biotechnology industry, particularly for the manufacture of therapeutic pDNA for gene therapy and DNA vaccination. Following fermentation, the cell paste is harvested and the plasmid DNA is extracted, typically using alkaline lysis. This production process differs substantially from recombinant protein expression in *E. coli*.

SHENTEK® *E. coli* (K-12) HCP ELISA Kit is specifically designed for the quantitative detection of *E. coli* (K-12) host cell proteins (HCPs) following an alkaline lysis-based extraction. The quantitative HCP standards and capture antibodies have been thoroughly characterized. The coverage of the *E. coli* (K-12) HCP antibody was evaluated and validated through 2D electrophoresis and LC-MS/MS analyses.

The assay performance meets the requirements of both bioprocess development and QC release testing, thereby eliminating the need to develop a customized or process-specific HCP assay for *E. coli* (K-12) - derived plasmid DNA production.

Kit manufacturing is carried out in compliance with ISO 13485 quality management system requirements, providing reliable and consistent performance for routine HCP monitoring throughout the pDNA manufacturing process.

| Product Number | Product Name                                                       | Quantity |
|----------------|--------------------------------------------------------------------|----------|
| 1301302        | SHENTEK® <i>E. coli</i> (K-12 & Alkaline Lysis) HCP ELISA Kit      | 96 tests |
| 1301302-Ab01   | SHENTEK® Anti- <i>E. coli</i> (K-12 & Alkaline Lysis) HCP Antibody | 1 mg     |



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For Research Use Only.

Not for diagnostic or therapeutic use.

## Key Features

- ✓ **Assay type:** Sandwich ELISA format
- ✓ **Linear range:** 3 to 729 ng/mL,  $R^2 > 0.990$
- ✓ **Limit of quantitation (LOQ):** 3 ng/mL
- ✓ **Precision (Repeatability):** CV < 15%
- ✓ **Accuracy:**  $80\% \leq \text{Recovery} \leq 120\%$
- ✓ **Specificity:** No cross-reactivity with different host cell proteins (e.g., Vero, HEK293T, Hensenula and CHO)
- ✓ **Robustness:** Consistent assay performance across different sample matrices, and consistent sample linearity throughout a dilution series
- ✓ **Stability:** Highly consistent product quality within and among batches for reliable QC results
- ✓ **Coverage:**
  - Risk factors of immunogenicity: High-risk *E. coli* (K-12) HCPs are covered in ELISA standards and antibodies
  - Coverage assessment: Coverage of the *E. coli* (K-12) HCP antibodies to the standards was validated by orthogonal methods of IMBS®-2D and IMBS®-LC/MS.

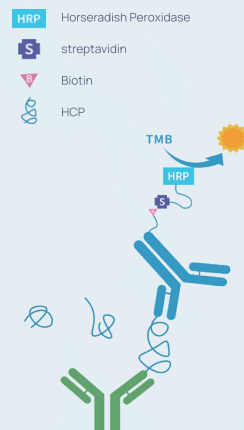


Figure 1 Diagram of Sandwich ELISA

## *E. coli* (K-12) HCP Standard & Antibody Coverage Analysis by IMBS®-2D & IMBS®-LC/MS

- ✓ Orthogonal proteomic methods to assess anti-HCP antibody coverage
- ✓ Process-specific and high-risk HCP analyses to ensure accurate and specific detection by ELISA
- ✓ Comprehensive study of coverage and specificity of the HCP standards to ensure reliability of immunoassays

Figure 2 SHENTEK® Capture Antibody Coverage Assessment Platform

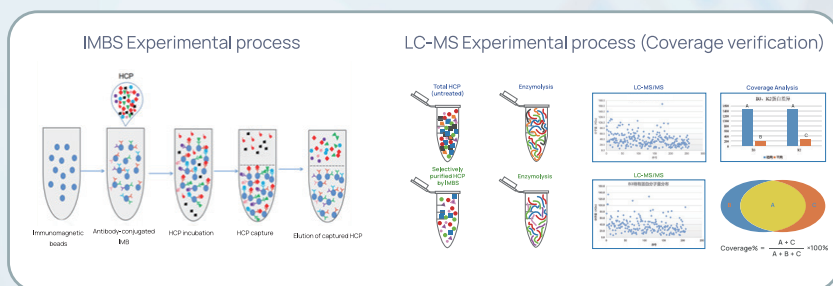
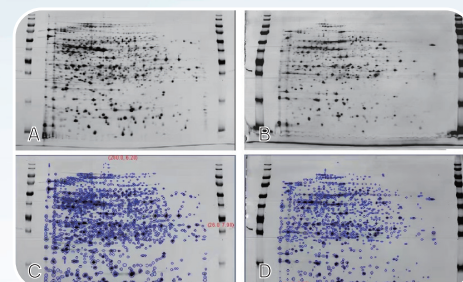


Figure 3 Coverage Assessment of *E. coli* (K12)-HCP Antibody by 2D analysis (Top row left A & right B, bottom row left C & right D)



## Assay difference between SHENTEK® *E. coli* (K-12) HCP ELISA Kit & Generic *E. coli* HCP ELISA Kit

An experimental study demonstrated a substantial difference between the HCP detection results between a generic *E. coli* HCP ELISA kit and an alkaline lysis extraction-specific *E. coli* (K-12) HCP ELISA kit (Figure 4).

Alkaline lysis causes extensive denaturation of cellular proteins, facilitating their subsequent separation from plasmid DNA during downstream purification. This denaturation can alter the structure and immunoreactivity of residual HCPs, potentially reducing their recognition by capture antibodies used in generic *E. coli* HCP ELISA kits. As a result, generic assays may yield lower apparent HCP levels and potentially underestimate residual HCP contamination in plasmid DNA products.

In contrast, the process-specific assay is developed using an immunogen and reference standard representative of the *E. coli* (K-12) HCP profile generated after the alkaline lysis extraction process. The corresponding antibodies are therefore better suited to recognizing process-relevant HCPs. This makes the process-specific assay more appropriate for the sensitive and reliable monitoring of residual HCPs throughout plasmid DNA manufacturing.

### *E. coli* HCP ELISA Assays for Alkaline Extracted pDNA Sample

(HCP ratio = 280 µg/mg)

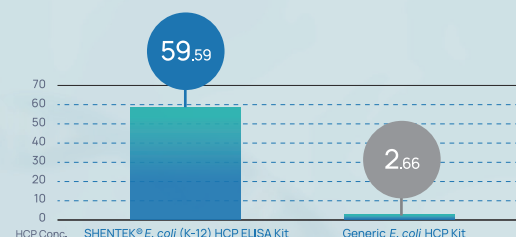


Figure 4. Test value of HCP Conc. (ng/mL) in pDNA sample from *E. coli* (K-12) expression and alkaline lysis extraction