

E.coli (K-12) HCP ELISA Kit

Product Introduction

Escherichia coli K-12 strains and their derivatives (DH5a, TOP10, JM109, etc.) are the main hosts for bacterial plasmid DNA (pDNA) production in the biotechnology industry, particularly therapeutic pDNA generation for gene therapy and DNA vaccination. Cell paste generated from fermentation is harvested and the plasmid extracted from the cell paste, typically via alkaline lysis, which is different from that of recombinant protein expression in Escherichia coli.

HZSKBIO® designed SHENTEK® E.coli (K-12) HCP ELISA kit, expressly for the quantitative measurement of HCP contamination of plasmid DNA samples from E. coli (K-12) expression and alkaline extraction. The quantitation standards and capture antibodies were characterized, and the coverage of E.coli (K-12) HCP antibodies to the antigen was validated by 2D and LC-MS/MS analyses. The assay performance met not only bioprocess development needs, but also that of QC release test, eliminating the need to develop a custom or process-specific assay. Kit manufacturing complies with ISO13485 quality standard.

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



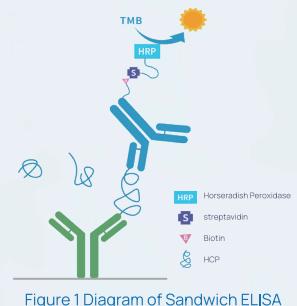
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Key Features

- ✓ 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 Recovery \leq 120%
- ✓ Specificity: No cross-reactivity with different host cell proteins (e.g., Vero, HEK293T, Hensenua 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.



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 HZSKBIO® Capture Antibody Coverage Assessment Platform

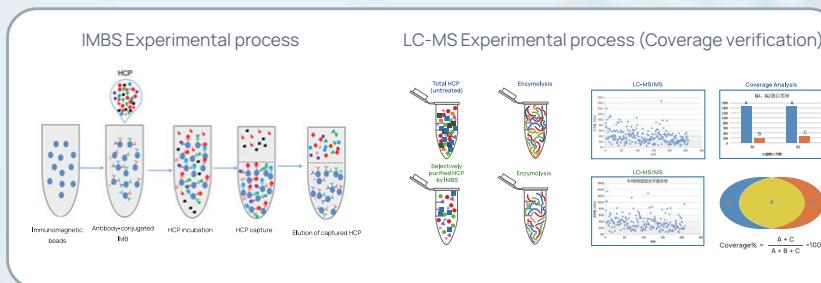
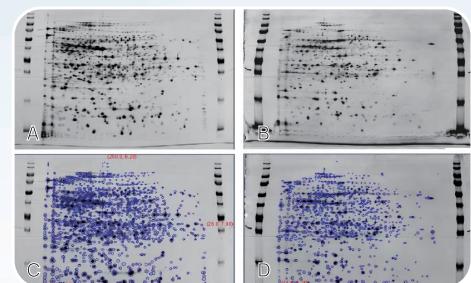


Figure 3 Coverage Assessment of E.coli (K12)-HCP Antibody by 2D analysis (Top row left A & right B, bottom row left C & right D)



A & C: HCPs sample, 100ug;
B & D: HCPs sample isolated by IMBS, 100ug, 2D Page, pH 5-8, 18cm

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

Experimental study illustrated a huge difference between the assay results of a generic E.coli HCP ELISA kit and the process-specific kit for E.coli K-12 expression together with alkaline extraction for plasmid DNA production (see Figure 4). Alkaline lysis denatures most of the proteins in the cells, which helps with the separation of the proteins from the plasmid later in the process. This step weakens the binding interactions between residual HCPs and the capture antibodies prepared in the generic E.coli HCP ELISA kits, resulting in lower HCP test values and potential immunogenicity risk in the final products. Thus the process-specific assay is appropriate in that the immunogen/standard and antibodies have been prepared from a set of HCPs unique to this isolation process, and the assay is specifically applicable to the monitoring of the process HCPs in plasmid manufacturing.

E.coli HCP ELISA Assays for Alkaline Extracted pDNA Sample

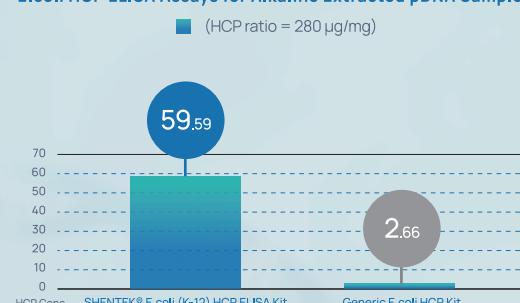


Figure 4. Test value of HCP Conc. (ng/mL) in pDNA sample from E. coli (K-12) expression and alkaline lysis extraction