Creation of a Broadly Reactive *E. coli* HCP Detection ELISA Suitable for Both B and K-12 Strain Platforms

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ABSTRACT

ELISA is a workhorse method of measuring host cell protein (HCP) impurities throughout downstream bioprocessing, monitoring HCP removal and thereby helping to ensure consistency in manufacturing of biopharmaceutical products. ELISA also acts in conjunction with, and orthogonally to, other analytical methods such as 2D Western blot and mass spectrometry.

Escherichia coli (*E. coli*) is among the most commonly used host organisms for scientific research and biotechnological applications, with approximately 30% of biopharmaceuticals being produced in *E. coli*. With respect to biotechnology, there are two major *E. coli* strain types which have application specific advantages. *E. coli* B strains have been optimized for fast growth and highly controllable protein expression and are most often used for recombinant protein expression. *E. coli* K-12 strains have greater genetic stability but lack some features for protein expression and are thus most commonly used for the generation of plasmids to support gene therapy programs. The use of bacterial cells, such as *E. coli*, in the generation of therapeutic biologics and genetic material requires the monitoring of impurities, which can include host cell proteins, host cell DNA, as well as growth media components.

Here we present the development of a new *E. coli* HCP antibody reagent and the corresponding validated ELISA for detection of *E. coli* HCP impurities in downstream bioprocessing. This *E. coli* HCP ELISA has been qualified for monitoring HCPs at all steps in the bioprocessing workflow for both recombinant proteins (produced by *E. coli* B strains) and for plasmid production that support gene therapies (produced by *E. coli* K-12 strains).

ASSAY QUALIFICATION PARAMETERS

In developing an HCP assay, such as the Rockland AccuSignal™ *E. coli* HCP ELISA, we always ensure that the following criteria are robustly tested and meet our rigorous specifications.

- Comprehensive HCP Coverage
- Wide Dynamic Range
- High Sensitivity (LLOQ and LOD)
- Low Intra- and Inter- Assay Variation
- Linearity and Sample Compatibility
- Wide Buffer Compatibility
- Reagent Stability

COMPREHENSIVE *E. COLI* HCP COVERAGE

A) DIBE Coverage

≥ 90% Antibody Coverage to *E. coli* B and K-12 Strain Antigens

E. coli Sample	Strain	Coverage (%)
DH5α	K-12	94
Origami2	K-12	90
Rosetta	B	94

B) ELISA Testing

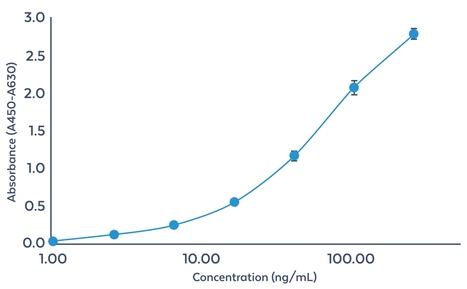
Both *E. coli* B and K-12 Strains are detected by ELISA

B or K-12	Specific Strain	HCP Detected in Assay
B	BL21 (DE3)*	YES
B	Rosetta	YES
K-12	Origami2	YES
K-12	DH5α	YES
K-12	XL1-Blue	YES

*The Kit's standard curve was generated against *E. coli* BL21 (DE3) cells.

Tables 1A & 1B. A pool of polyclonal antibody sera was selected that showed the broadest reactivity between antibody and antigen. A) Antibody coverage analysis was assessed by DIBE, a type of 2D gel electrophoresis and Western blot assay, with the antibodies selected for this kit shown to have a greater than 90% reactivity for HCPs from both K and B strains of *E. coli*. B) Spike samples of five B and K strains were successfully detected by the ELISA, demonstrating its multi-strain utility.

WIDE DYNAMIC RANGE



Assay Range:
2.6 – 250 ng/mL

Recovery:
$R^2 > 0.99$

Figure 2. The protein standard provided in the kit was utilized to construct a standard curve spanning from 250 to 2.6 ng/mL This curve was subsequently meticulously measured in triplicate across six distinct assays, resulting in a total of eighteen replicates. The assay successfully validated the effectiveness of this broad range (2.6 to 250 ng/mL) and demonstrated its remarkable ability to accurately interpolate its calibrators (Recovery %) with an exceptionally strong goodness of fit ($R^2 > 0.99$).

HIGH SENSITIVITY

A) LLOQ

E. coli HCP Concentration	4 ng/mL	3 ng/mL	2 ng/mL
Mean Interpolated Concentration (ng/mL)	3.4	2.5	1.6
Recovery (%)	83.8	82.4	78.9
CV (%)	14.9	17.4	18.0

Lower Limit of Quantification (LLOQ):
3 ng/mL

B) LOD

Parameter	Average
OD of Blank	0.029
σ of Blank	0.002
OD of Blank + 3σ (LOD)	0.034
OD at 2.6 ng/mL	0.102
Back-Calculated Value of OD + 3σ (LOD)	≤ 1.0 ng/mL*

Limit of Detection (LOD):
≤ 1 ng/mL

*A precise value cannot be calculated because the OD of the Blank + 3σ falls below the 4PL calculations.

Tables 2A & 2B. A) The Lower Level of Quantification (LLOQ) is the lowest concentration of antigen we could reliably detect within a 70 – 130 % recovery and a %CV of less than 20%. The LLOQ was identified to be 3 ng/mL. B) The Limit of Detection (LOD) is the lowest concentration that is detectable from the background and is defined as the mean absorbance of the blank plus three standard deviations (σ). A Lower Limit of Detection less than 1 ng/mL was identified.

PARAMETERS OF ASSAY

AccuSignal™ *E. coli* HCP ELISA Kit

KJB-4003

Specifications	Parameter
Limit of Detection (LOD)	≤ 1.0 ng/mL
Lower Limit of Quantification (LLOQ)	3.0 ng/mL
Range	2.6 – 250.0 ng/mL
Precision (Intra-Assay Variability)	≤ 20%
Precision (Inter-Assay Variability)	≤ 25%

LOW INTRA AND INTER-ASSAY VARIATION

Intra-assay Variability: Average CV% of 5%

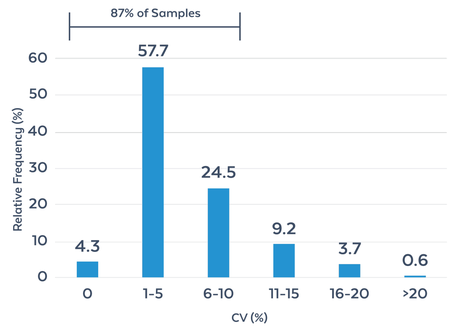


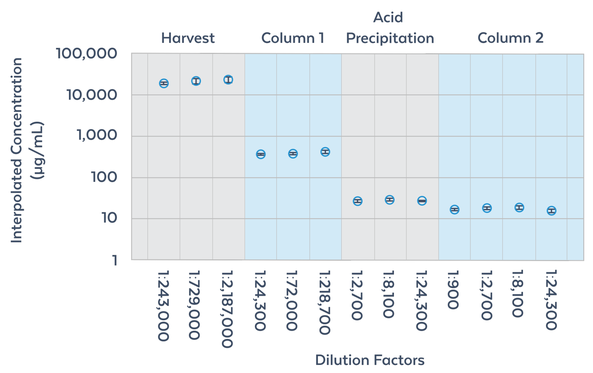
Figure 3. To analyze intra-assay variation, the CV% from the interpolated concentration of 9 in-process samples was tested in triplicate dilutions across 35 separate plates. From these results, 163 triplicate dilutions fell within the measurable range of the standard curve and were evaluated for CV%. Of these, 87% exhibited an intra-assay CV% less than 10%, with an average CV% of 5%.

Inter-assay Variability: CV% < 10%

Spiked HCP Concentration (ng/mL)	Measured HCP (ng/mL)	CV (%)	Recovery (%)
100	99.2	3.1	99.3
25	24.5	4.4	97.9
5	5.5	7.3	103.4

Table 3. To measure inter-assay precision, four plates were spiked with concentrations of *E. coli* HCP at 100 ng/mL, 20 ng/mL, and 5 ng/mL, each replicated four times (a total of 16 replicates). To calculate the inter-assay precision, the means of the OD were determined along with the mean Recovery % and CV%. The data reveal CV% not exceeding 10% across the four plates tested.

LINEARITY AND SAMPLE COMPATIBILITY



Dilutional linearity and parallelism observed for all dilutions and HCP purification stages

Figure 4. Each bioprocess step should reproducibly decrease the amount of HCPs present, demonstrating control of the purification process. This *E. coli* HCP ELISA Kit showcases its capability to monitor HCP levels throughout a purification process and additionally exhibits excellent dilutional linearity and parallelism between dilution steps, ensuring consistent results across a broad range of dilutions and proving its utility for bioprocess manufacturing.

WIDE BUFFER COMPATIBILITY

Compatible with buffers at varying pH and salt concentrations

Matrix Buffer	Minimum Matrix Dilution Factor	Recovery (%)	CV (%)
50 mM Tris, pH 8.0	2.1	112	7
50 mM Sodium Phosphate, 0.3 M NaCl, 0.5 M Imidazole, pH 8.0	4.3	80	2
25 mM Sodium Acetate, pH 2.5	32	103	6
25 mM Citric Acid, 0.5 M NaCl, pH 2.0	32	91	3

Table 4. Biomanufacturing processes require a variety of buffers for different purification stages. Multiple buffers were tested at varying salt concentrations and pH levels. In-specification recovery from a diverse matrix of buffers was demonstrated, showing the ELISA Kit exhibits excellent sample compatibility throughout the bioprocess.

REAGENT STABILITY

Predicted Shelf-Life:
24-Months at 4°C

Parameter	Pass Criteria	Result (Day 187 at 25°C)
Absorbance of 100 ng/mL Standard	≥ 1.0	PASS
Absorbance of blank standard	≤ 0.1	PASS
Intra-Assay CV	≤ 20%	PASS
Recovery of each protein standard	80 – 120%	PASS
LOD (ng/mL)	≤ 1.0 ng/mL	PASS

Table 5. An accelerated stability study was conducted to validate a shelf life of 24 months. According to the Variable Q_{10} Method, an accelerated study conducted at 25 °C over 187 days is equivalent to 24 months at the intended storage condition of 2-8 °C. Therefore, the kit components were stored at 25 °C, and assay performance was evaluated at regular intervals over 187. Notably, all criteria were met, which successfully validated the shelf life of 24 months for this kit.