Validation Summary of

E.coli (Protein Expression Strains) HCP ELISA Kit

(One-step ELISA)

■ INTRODUCTION

This report summarizes assay performance of SHENTEK® *E.coli* (Protein Expression Strains) HCP ELISA Kit (One-step ELISA). The kit is manufactured by Huzhou Shenke Biotechnology Co., Ltd. The data of this summary is for reference use. To demonstrate that the kits are suitable for an intended purpose, appropriate validation or qualification study with actual biological sample should be considered.

Parameters that may be evaluated for method validation are linearity, range, quantitation limit (QL), detection limit (DL), specificity, precision, accuracy, antibody coverage and robustness.

The report may be appropriate for actual biological sample on a case-by-case basis, and the users could consider completing sample suitability test (including QL and specificity validation) or more to meet regulatory requirements.

■ MATERIALS & METHODS

1. SHENTEK® *E.coli* (Protein Expression Strains) HCP ELISA Kit (One-step ELISA), Product No. 1301301-1.
2. The production of the kit is compliant with the requirements of ISO13485.
3. The assay validation compliant with the pharmacopoeia requirement (e.g., ICH Q2R2, USP<1132>, EP<2.6.34>). Please refer to the reference for details.

■ RESULTS

1. Linearity and range

The assay range of the kit is 1.5-243 ng/mL, and $R^2 \geq 0.990$. The CV and the relative bias of the highest and lowest concentration points are no more than 25%. The CV and the relative bias of the remaining concentration points are no more than 20%.

Table 1. Linearity and range results

Theoretical Conc. (ng/mL)	Ave.value(ng/mL)	CV (%)	Relative bias (%)
243	244.64	11.0	0.7
81	81.10	7.3	0.1
27	27.06	1.9	0.2
9	8.92	10.7	0.9
3	3.08	3.5	2.6
1.5	1.47	6.9	2.1
R^2	1.00000		

2. Quantitation limit (QL)

The lower quantitation limit (LLOQ) of the assay is 1.5 ng/mL, and the upper quantitation limit (ULOQ) is 243 ng/mL. The CV and the relative bias are no more than 25%.

Table 2. Quantitation limit results

Theoretical Conc. (ng/mL)	243	1.5
Ave. value (ng/mL)	231.40	1.58
CV(%)	11.2	11.7
Relative bias (%)	4.8	5.3

3. Detection limit (DL)

The detection limit was defined as the detection concentration corresponding to the average value of the blank +2SD, and the detection limit of the kit is 1.19 ng/mL.

4. Accuracy

The quality control samples (QCs) were prepared at 5 concentration levels within the calibration curve range: LLOQ (Conc. 1.5 ng/mL), low QC (Conc. 4.5 ng/mL), medium QC (Conc. 121.5

ng/mL), high QC (Conc. 194 ng/mL) and ULOQ (Conc. 243 ng/mL).

The relative bias for ULOQ and LLOQ are no more than 25% and for others are no more than 20%. The recovery rate is 75%-125% for LLOQ and ULOQ samples, and 80-120% for all other samples.

Table 3. Accuracy results

QCs	Sample (ULOQ) n=3	Sample (high) n=3	Sample (medium) n=3	Sample (low) n=3	Sample (LLOQ) n=3
Theoretical Conc. (ng/mL)	243	194.4	121.5	4.5	1.5
Ave. value (ng/mL)	203.74	165.63	114.97	4.89	1.65
Relative bias (%)	16.2	14.8	5.4	8.7	10.2
Recovery rate (%)	83.8	85.2	94.6	108.7	110.2

5. Precision

5.1 Repeatability

Samples with three concentration points were tested for 10 times respectively, CV values were no more than 20%.

Table 4. Repeatability results

QCs	Sample (high)	Sample (medium)	Sample (low)
Theoretical Conc. (ng/mL)	194.4	121.5	4.5
Ave. value (ng/mL)	165.98	107.27	4.40
CV (%)	7.1	8.6	4.5

5.2 Intermediate precision

Samples at five concentration points were tested by 3 technicians in 3 independent experiments. For ULOQ and LLOQ, the CV value was no more than 25%, for high, medium and low samples, the CV value was no more than 20%.

Table 5. Intermediate precision results

QCs	Sample (ULOQ) n=9	Sample (high) n=9	Sample (medium) n=9	Sample (low) n=9	Sample (LLOQ) n=9
Theoretical Conc. (ng/mL)	243	194.4	121.5	4.5	1.5
Ave. value (ng/mL)	232.38	178.05	118.97	5.13	1.65
CV (%)	14.1	9.5	5.2	9.1	14.0

5.3 Batch-to-batch precision

Three batches of the kit were tested in 3 separate assays to assess batch-to-batch precision, the CV value was no more than 20%.

Table 6. Batch-to-batch precision results

QCs	Sample (high)			Sample (medium)			Sample (low)		
Batch	1 n=9	2 n=9	3 n=9	1 n=9	2 n=9	3 n=9	1 n=9	2 n=9	3 n=9
Theoretical Conc. (ng/mL)	194.4			121.5			4.5		
Ave. value (ng/mL)	181.97			122.21			4.94		
CV (%)	10.6			10.0			9.4		

6. Specificity

6.1 Specificity for HCP

The HCPs of common engineering cell lines preparation and detection assay were carried out for spiked concentrations, and recovery rate were analyzed. The mean value of detection assay of the engineering cell lines (test Conc. 243 ng/mL) were lower than the LLOQ.The recovery rates of spiked samples with different concentrations were 80%-120%. The results showed no cross-reactivity to the assay.

Table 7. Specificity results

Host Cell Proteins	Ave. value (ng/mL)	Spiked Conc. (ng/mL)	Recovery rate (%)
Vero HCP	Undet.	4.5	93.7
		194.4	95.2
293T HCP	Undet.	4.5	97.0
		194.4	96.5
CHO HCP	3.89×10^{-1}	4.5	103.0
		194.4	102.9

6.2 Selectivity (Matrix effect)

The mean value of the detection was less than the LLOQ, and the recovery rate was 80%-120%.

The samples with pH between 6.0 and 8.5 showed no interference effect to the assay.

Table 8. Matrix interference results

Sample matrix	20 mM PB, pH 6.0	20 mM PB, 100 mM NaCl, pH 7.0	20mM Tris, 0.05M $(NH_4)_2SO_4$, pH 8.5
Dilution ratio	2	NA	NA
Spiked Conc. (ng/mL)	1.5		
Ave. value (ng/mL)	7.71×10^{-1}	9.71×10^{-1}	7.38×10^{-1}
Recovery rate (%)	107.0	100.2	92.9

6.3 Antibody coverage

The HCP antibody coverage of *E.coli* (Protein Expression Strains) HCP ELISA Kit (One-step ELISA) was evaluated by Immunomagnetic Bead Separation (IMBS) method combined with 2D SDS-PAGE (IMBS-2D) and LC-MS (IMBS-LC-MS) analysis.

The antibody coverage obtained by IMBS-2D method was 70.6%-100%.

The antibody coverage obtained by IMBS-LC-MS method was 88.8%.

7. Robustness

7.1 Incubation condition

The assay is designed to incubate at 25±5℃. Both CV and relative bias were no more than 20% .

Table 9. Robustness results-Incubation temperature

Temperature	20℃		25℃		30℃	
QCs	Sample (low) n=3	Sample (high) n=3	Sample (low) n=3	Sample (high) n=3	Sample (low) n=3	Sample (high) n=3
Theoretical Conc (ng/mL)	4.5	194.4	4.5	194.4	4.5	194.4
Ave. value (ng/mL)	4.43	178.94	4.89	165.63	4.49	213.97
CV (%)	3.8	10.9	6.2	13.9	5.8	8.1
Relative bias (%)	1.5	8.0	8.7	14.8	0.1	10.1

7.2 Instrument Suitability

7.2.1 Microplate Reader

The kit is applicable to but not limited to the following instruments. Both CV and relative bias were no more than 20% .

Table 10. Instrument suitability results - Microplate Reader

Microplate Readers	Thermo Multiskan FC		Bio-Tek Synergy2	
QCs	Sample (Low) n=3	Sample (High) n=3	Sample (Low) n=3	Sample (High) n=3
Theoretical Conc. (ng/mL)	4.5	194.4	4.5	194.4
Ave. value (ng/mL)	4.16	181.66	4.56	174.76
CV (%)	9.8	4.1	8.7	4.3
Relative bias (%)	7.5	6.6	1.3	10.1

7.2.2 Microplate Washer

The kit is suitable for automatic washing. Both CV and relative bias were no more than 20% .

Table 11. Instrument suitability results - Microplate Washer

Mode	Automatic washing	
QCs	Sample (Low) n=3	Sample(High) n=3
Theoretical Conc. (ng/mL)	4.5	194.4
Ave. value (ng/mL)	4.48	198.39
CV (%)	11.5	8.3
Relative bias (%)	0.3	2.1

■ CONCLUSION

Parameters concluding linearity, range, QL, DL, specificity, precision, accuracy, and robustness were all evaluated and met the requirements.

■ REFERENCES

[1] ICH Q2 (R2) VALIDATION OF ANALYTICAL PROCEDURES

[2] USP <1132> Residual Host Cell Protein Measurement in Biopharmaceuticals

[3] EP <2.6.34> HOST-CELL PROTEIN ASSAYS

[4] ChP <9012> Guidance for method validation of quantitative analysis of biological samples

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