Validation Summary of

E.coli (K-12 & Alkaline Lysis) HCP ELISA Kit

■ INTRODUCTION

This report summarizes assay performance of SHENTEK® *E.coli* (K-12 & Alkaline Lysis) HCP ELISA Kit. 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* (K-12 & Alkaline Lysis) HCP ELISA Kit, Product No. 1301302.
2. The production of the kit is compliant with the requirements of ISO13485.
3. The assay validation compliant with the pharmacopoeia requirement (e.g., 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 3-729 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 (%)
729	731.28	13.4	0.3
243	247.46	12.0	1.8
81	77.34	9.7	4.5
27	29.29	4.5	8.5
9	8.53	1.0	5.2
3	2.97	1.8	1.0
R^2	0.99967		

2. Quantitation limit (QL)

The lower quantitation limit (LLOQ) of the assay is 3 ng/mL, and the upper quantitation limit (ULOQ) is 729 ng/mL. The CV and the relative bias are no more than 25%.

Table 2. Quantitation limit results

Quantitation limit	ULOQ			LLOQ	
Theoretical Conc. (ng/mL)	729	486	243	6	3
Ave. Value (ng/mL)	690.34	433.11	224.60	5.76	2.78
CV (%)	5.4	5.1	9.4	5.5	5.6
Relative bias (%)	5.3	10.9	7.6	3.9	7.4

3. Detection limit (DL)

The detection limit is defined as the detection concentration corresponding to the average value of the blank +2SD, and the detection limit of the kit is 1.26 ng/mL.

4. Accuracy

The quality control samples (QCs) were prepared at 5 concentration levels within the calibration

curve range: LLOQ (Conc. 3 ng/mL), low QC (Conc. 6 ng/mL), medium QC (Conc. 364.5 ng/mL), high QC (Conc. 583.2 ng/mL) and ULOQ (Conc. 729 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)	729	583.2	364.5	6	3
Ave. value (ng/mL)	728.80	580.83	317.34	5.74	3.20
Relative bias (%)	0.0	0.4	12.9	4.4	6.7
Recovery rate (%)	100.0	99.6	87.1	95.6	106.7

5. Precision

5.1 Repeatability

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

Table 4. Repeatability results

QCs	Sample (high)	Sample (medium)	Sample (low)
Theoretical Conc. (ng/mL)	583.2	364.5	6
Ave. value (ng/mL)	572.03	335.15	5.41
CV (%)	5.9	5.6	4.4

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 are no more than 25%, for high, medium and low samples, the CV value are 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)	729	583.2	364.5	6	3
Ave. value (ng/mL)	664.74	562.32	348.03	5.53	2.84
CV (%)	7.7	6.9	6.8	3.1	14.6

5.3 Batch-to-batch Precision

Three batches of the kit were tested in 3 separate assays to assess batch-to-batch precision. For ULOQ and LLOQ, the CV value are no more than 25%; for high, medium and low samples, the CV value are no more than 20%.

Table 6. Batch-to-batch precision results

QCs	Sample (ULOQ)			Sample (high)			Sample (medium)			Sample (low)			Sample (LLOQ)		
Batch	1 n=3	2 n=3	3 n=3	1 n=3	2 n=3	3 n=3	1 n=3	2 n=3	3 n=3	1 n=3	2 n=3	3 n=3	1 n=3	2 n=3	3 n=3
Theoretical Conc. (ng/mL)	729			583.2			364.5			6			3		
Ave. value (ng/mL)	604.73			551.35			330.42			5.70			2.97		
CV (%)	9.4			3.5			7.5			3.5			7.6		

6. Specificity

6.1 Specificity for HCP

The HCPs of common engineering cell lines (e.g., CHO, Vero, 293T, *Hansenula polymorpha,* test Conc. 3645 ng/mL) were prepared and detected, the mean value were all lower than the LLOQ.The results showed no cross-reactivity to the assay.

6.2 Selectivity (Matrix effect)

Two matrix samples (*i.e.* 0.075% PBST, pH 6.0 and 0.075% PBST, pH 7.4, 0.01% SDS) showed no interference effect to the assay, and the recovery rate was 80%-120%.Three Matrix samples (*i.e.* 10 mM Tris-HCl,1 mM EDTA, pH 8.0； 0.075% PBST, pH 7.4， 0.05% SDS and 0.075% PBST, pH 8.0) showed no interference effect to the assay after diluted 2-8 times, and the recovery rate was 80%-120%.

Table 7. Matrix interference results

Sample matrix	Dilution ratio	Recovery rate (%)
0.075% PBST, pH 6.0	NA	87.4
0.075% PBST, pH 7.4, 0.01% SDS	NA	80.8
10 mM Tris-HCl, 1 mM EDTA, pH 8.0	2	89.2
0.075% PBST, pH 7.4, 0.05% SDS	4	90.1
0.075% PBST, pH 8.0	8	93.3

6.3 Antibody coverage

The HCP antibody coverage of *E.coli* (K-12 & Alkaline Lysis) HCP ELISA Kit 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 70.0%-80.6%.

The antibody coverage obtained by IMBS-LC-MS method was 85.5%.

7. Robustness

7.1 Incubation condition

The assay is designed to incubate at 25±3℃. The suitable speed of microplate thermostatic oscillator for incubation is at 300-500 rpm. Two key reagents Anti-E.coli HCP-AC: Biotinylated Conjugate and Streptavidin-HRP were added at 95 μL-105 μL/well, respectively, and had no effect on the stability of the detection system.

For high Sample, both CV and relative bias are no more than 20%, for LLOQ, both CV and relative bias are no more than 25%.

Table 8. Robustness results-Incubation temperature

Temperature	22℃		25℃		28℃	
QCs	Sample (high) n=3	Sample (LLOQ) n=3	Sample (high) n=3	Sample (LLOQ) n=3	Sample (high) n=3	Sample (LLOQ) n=3
Theoretical Conc. (ng/mL)	583.2	3	583.2	3	583.2	3
Ave. value (ng/mL)	509.37	2.73	580.50	2.92	540.21	3.34
CV (%)	4.7	7.8	10.2	6.7	5.3	2.3
Relative bias (%)	12.7	8.8	0.5	2.7	7.4	11.2

Table 9. Robustness results-Incubation speed

Incubation speed	300 rpm		400 rpm		500 rpm	
QCs	Sample (high) n=3	Sample (LLOQ) n=3	Sample (high) n=3	Sample (LLOQ) n=3	Sample (high) n=3	Sample (LLOQ) n=3
Theoretical Conc. (ng/mL)	583.2	3	583.2	3	583.2	3
Ave. value (ng/mL)	583.76	2.79	580.50	2.92	534.91	3.39
CV (%)	1.1	1.1	10.2	6.7	9.4	7.6
Relative bias (%)	0.1	6.8	0.5	2.7	8.3	13.1

Table 10. Robustness results-Key reagent addition volume

Key reagent addition volume	95 μL		100 μL		105 μL	
QCs	Sample (high) n=3	Sample (LLOQ) n=3	Sample (high) n=3	Sample (LLOQ) n=3	Sample (high) n=3	Sample (LLOQ) n=3
Theoretical Conc. (ng/mL)	583.2	3	583.2	3	583.2	3
Ave. value (ng/mL)	565.38	3.03	555.47	2.82	572.83	3.10
CV (%)	1.4	0.4	9.2	4.5	8.1	2.5
Relative bias (%)	3.1	1.0	4.8	6.0	1.8	3.3

7.2 Instrument Suitability

7.2.1 Microplate Reader

The kit is applicable to but not limited to the following instruments. For high Sample, both CV and relative bias are no more than 20%, for LLOQ, both CV and relative bias are no more than 25%.

Table 11. Instrument suitability results - Microplate Reader

Microplate Readers	Thermo Multiskan FC		Bio-Tek Synergy2	
QCs	Sample (high) n=3	Sample (LLOQ) n=3	Sample (high) n=3	Sample (LLOQ) n=3
Theoretical Conc. (ng/mL)	583.2	3	583.2	3
Ave. value (ng/mL)	547.16	3.22	506.44	3.12
CV (%)	12.8	3.7	2.4	4.7
Relative bias (%)	6.2	7.2	13.2	4.1

7.2.2 Microplate Washer

The kit is suitable for automatic washing. For high Sample, both CV and relative bias are no more than 20%, for LLOQ, both CV and relative bias are no more than 25%.

Table 12. Instrument suitability results - Microplate Washer

Mode	Automatic washing	
QCs	Sample (high) n=3	Sample (LLOQ) n=3
Theoretical Conc. (ng/mL)	583.2	3
Ave. value (ng/mL)	502.04	2.84
CV (%)	3.9	4.5
Relative bias (%)	13.9	5.5

■ 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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