Validation Summary of

Sf9 HCP ELISA Kit (One-step ELISA)

■ INTRODUCTION

This report summarizes assay performance of SHENTEK® Sf9 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), specificity, precision, accuracy, and robustness, etc..

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® Sf9 HCP ELISA Kit(One-step ELISA), Product No. 1301312
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 3-243 ng/mL,and $R^2 \geq 0.990$. The CV of the highest and lowest concentration points is no more than 25%, and the relative bias is within ±25%; CV of the remaining concentration points is no more than 20%, and the relative bias is within ±20%.

Table 1. Linearity and range results

Theoretical Conc. (ng/mL)	Ave.value (ng/mL)	CV (%)	Relative bias (%)
243	243.02	2.4	0.01
81	80.99	2.9	-0.01
27	27.04	1.7	0.2
9	8.99	2.9	-0.1
3	2.90	6.2	-3.3
R^2	4-PL, 0.999		

2. Quantitation limit (QL)

The lower quantitative limit (LLOQ) of the assay is 3 ng/mL, and the upper quantitative limit (ULOQ) is 243 ng/mL. The CV is no more than 25% and the relative bias is within ±25%.

Table 2. Quantitative limit results

Theoretical Conc. (ng/mL)	CV (%)	Relative bias (%)
3 (n=10)	10.9	-2.1
243 (n=10)	2.4	0.3

3. Detection limit (DL)

The detection limit was defined as the detection concentration corresponding to the average value (n=10) of the blank +2SD, and the detection limit of the kit is 0.9 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. 100 ng/mL), High QC (Conc. 182 ng/mL) and ULOQ (Conc. 243 ng/mL).

The recovery rate is 75%-125% for LLOQ and ULOQ samples, and 80-120% for 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	182	100	6	3
Ave. Value (ng/mL)	244.48	176.53	93.56	5.64	2.67
Recovery Rate (%)	100.6	97.0	93.6	94.0	88.9

5. Repeatability

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

Table 4. Repeatability results

QCs	Sample (High) n=10	Sample (Medium) n=10	Sample (Low) n=10
Theoretical Conc. (ng/mL)	182	100	6
Ave. Value (ng/mL)	182.79	98.55	5.97
CV (%)	3.2	4.3	7.7

6. Specificity

6.1 Specificity for HCP

The HCPs of commonly used cell lines were prepared at 2.5 μg/mL in calibration standard diluent and assayed for cross-reactivity. The results showed no cross-reactivity to the assay.

Table 5. Specificity results

Host Cell Proteins	Ave. Value (ng/mL)	Spiked Conc. (ng/mL)	Recovery Rate (%)
CHO HCP	< LLOQ	5	96.4
E.coli BL21 HCP	< LLOQ	5	100.0
MDCK HCP	< LLOQ	5	102.9
Vero HCP	< LLOQ	5	110.6
P.pastoris HCP	< LLOQ	5	110.4
HEK293T HCP	< LLOQ	5	95.0

6.2 Selectivity

The recovery of Sf9 HCP spiked to 3 ng/mL (LLOQ) in commonly used matrices was evaluated. The tested matrices showed no interference to the assay.

Table 6. Selectivity results

Sample matrix	Spiked Conc. (ng/mL)	Recovery Rate (%)
PB Buffer, pH 6.0	3	123.7
PB Buffer, pH 8.5	3	89.3
Tris Buffer, pH8.5	3	77.2
Citrate Buffer, pH5.5	3	77.4

6.3 Antibody coverage

The HCP antibody coverage of Sf9 HCP ELISA Kit (One-step ELISA) was evaluated by Immunomagnetic Bead Separation (IMBS) method combined with 2D-PAGE (IMBS-2D) or LC-MS (IMBS-LC-MS) analysis.

The antibody coverage obtained by IMBS-2D method was 70.4%-96.0%.

The antibody coverage obtained by IMBS-LC-MS method was 91.2%（Unique Peptide ≥2）.

7. Robustness

7.1 Incubation condition

The assay is designed to conducted at 25℃±5℃. Data acquisition should no more than 30 minutes after color-developing termination. The CV is no more than 20% and the relative bias is within ± 20%.

Table 7. 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)	6	182	6	182	6	182
Ave. Value (ng/mL)	6.52	180.32	6.81	184.14	7.12	187.53
CV(%)	7.1	9.4	5.7	1.7	8.4	4.3
Relative bias (%)	8.6	-0.9	13.6	1.2	18.7	3.0

Table 8. Robustness results-Data acquisition time

Time	5 min		10 min		20 min		30 min	
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	Sample (Low) n=3	Sample (High) n=3
Theoretical Conc. (ng/mL)	6	182	6	182	6	182	6	182
Ave. Value (ng/mL)	6.81	184.14	7.05	181.97	6.99	182.24	7.06	184.55
CV(%)	5.7	1.7	11.9	0.5	11.9	0.5	11.7	0.5
Relative bias (%)	13.6	1.2	17.6	-0.02	16.4	0.1	17.7	1.4

7.2 Instrument Suitability

7.2.1 Microplate Reader

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

Table 9. Instrument suitability results - Microplate Reader

Microplate Readers	Thermo Multiskan FC		MD Spectra Max ABS	
QCs	Sample (Low) n=3	Sample (High) n=3	Sample (Low) n=3	Sample (High) n=3
Theoretical Conc. (ng/mL)	6	182	6	182
Ave. Value (ng/mL)	6.81	184.14	6.69	183.66
CV(%)	5.7	1.7	8.3	1.1
Relative bias (%)	13.6	1.2	11.5	0.9

7.2.2 Microplate Washer

The kit is suitable for automatic washing and manual washing. The CV is no more than 20% and the relative bias is within ±20%.

Table 10. Instrument suitability results - Microplate Washer

Methods	Manual		Thermo Scientific™ Wellwash™	
QCs	Sample (Low) n=3	Sample (High) n=3	Sample (Low) n=3	Sample (High) n=3
Theoretical Conc. (ng/mL)	6	182	6	182
Ave. Value (ng/mL)	6.63	177.92	6.81	184.14
CV(%)	9.8	4.5	5.7	1.7
Relative bias (%)	10.5	-2.2	13.6	1.2

■ CONCLUSION

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

■ REFERENCES

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

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

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

[4] ICH M10 on bioanalytical method validation

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

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