Validation Summary of

HEK293 HCP ELISA Kit（One-step ELISA）

■ INTRODUCTION

This report summarizes assay performance of SHENTEK® HEK293 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® HEK293 HCP ELISA Kit (One-step ELISA), Product No. 1301311.
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 6-540 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 (%)
6	5.13	5.2	-14.5
18	18.66	2.5	3.6
54	54.83	1.9	1.5
135	133.74	2.6	-0.9
270	270.74	1.4	0.3
540	539.87	1.7	0.0
R^2	0.99998		

2. Quantitation limit (QL)

The lower limit of quantification (LLOQ) of the assay is 6 ng/mL, and the upper limit of quantification (ULOQ) is 540 ng/mL. The CV was no more than 25% and the relative bias was within ±25%.

Table2. Quantitation limit results

Theoretical Conc. (ng/mL)	CV(%)	Relative bias (%)
6 (n=10)	3.4	1.9
540 (n=10)	3.0	-1.7

3. Detection limit (DL)

The detection limit was defined as the detection concentration corresponding to the average value (n=20) of the blank +2SD, and the detection limit of the kit is 1 ng/mL.

4. Accuracy

The quality control samples (QCs) were prepared at 5 concentration levels within the calibration curve range: LLOQ (Conc. 6 ng/mL), Low QC (Conc. 15 ng/mL), Medium QC (Conc. 216 ng/mL), High QC (Conc. 432 ng/mL) and ULOQ (Conc. 540 ng/mL).

The Recovery rate was 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)	540	432	216	15	6
Ave. Value (ng/mL)	535.95	427.52	222.27	14.50	5.97
Recovery Rate (%)	99.3	99.0	102.9	96.7	99.5

5. Precision

5.1 Repeatability

Precision was determined by analysing at least 10 replicates. The CV was 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)	432	216	15
Ave. Value (ng/mL)	429.56	217.16	14.51
CV(%)	2.4	3.7	3.0

5.2 Batch-to-batch precision

Two batches of the kit were tested in two separate assays to assess batch-to-batch precision. The CV was no more than 25% for LLOQ and ULOQ samples, and no more than 20% for all other samples.

Table 5. Batch-to-batch precision results

QCs	Sample (ULOQ) n=3		Sample (High) n=3		Sample (Medium) n=3		Sample (Low) n=3		Sample (LLOQ) n=3	
Batch	1	2	1	2	1	2	1	2	1	2
Theoretical Conc. (ng/mL)	540		432		216		15		6	
Ave. Value (ng/mL)	525.14	535.95	453.86	427.52	234.79	222.27	15.55	14.50	4.93	5.97
CV(%)	3.2		3.7		3.4		4.2		13.6	

6. Specificity

6.1 Specificity for HCP

The HCPs of commonly used cell lines were prepared at 5.4 μg/mL in calibration standard diluent and assayed for cross-reactivity.The average detection value of HCPs was no more than the LLOQ and recovery rate was 80%-120%. The results showed that HCPs of Sf9 and *P.pastoris* showed no cross-reactivity to the assay.

Table 6. Specificity results (for HCPs)

Host Cell Proteins	Ave. Value (ng/mL)	Spiked Conc. (ng/mL)	Recovery Rate (%)
Sf9 HCP	ND*	15	117.8
P.pastoris HCP	ND*	15	116.2

*ND: no data (OD value of sample was lower than the OD value of blank).

6.2 Selectivity (Matrix effect)

The recovery rate of HEK293 HCP spiked to 6ng/mL (LLOQ) in commonly used matrices was evaluated, the recovery rate was 75%-125%. The tested matrices showed no interference to the assay.

Table 7. Selectivity results

Sample matrix	Spiked Conc. (ng/mL)	Recovery Rate (%)
1×PBS, 0.075% Tween-20, 0.5% BSA, pH 6.0	6	91.1
Hanks' Balanced Salt Solution (HBSS) (2-fold dilution)	6	104.7

6.3 Antibody coverage

The HCP antibody coverage of HEK293 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 67.1%-82.8%.

The antibody coverage obtained by IMBS-LC-MS method was 79.7% (Unique peptide≥2).

7. Robustness

7.1 Incubation condition

The assay is designed to conducted at 25℃±3℃. The suitable speed for incubation is at 500-600 rpm. The CV was no more than 20% and the relative bias was within ±20%.

Table 8. Robustness results-Incubation condition

Temperature	22℃		25℃		28℃	
Incubation speed	500 rpm		500 rpm		600 rpm	
QCs	Sample (Low) n=4	Sample (High) n=4	Sample (Low) n=4	Sample (High) n=4	Sample (Low) n=4	Sample (High) n=4
Theoretical Conc. (ng/μL)	15	432	15	432	15	432
Ave. Value (ng/μL)	16.58	432.91	16.81	432.34	16.54	438.41
CV(%)	4.7	5.0	3.9	5.7	9.6	4.5
Relative bias (%)	10.6	0.2	12.1	0.1	10.3	1.5

7.2 Instrument Suitability

7.2.1 Microplate Reader

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

Table 9. Instrument suitability results - Microplate Reader

Microplate Readers	MD Spectra Max ABS		Thermo Multiskan FC	
QCs	Sample (Low) n=4	Sample (High) n=4	Sample (Low) n=4	Sample (High) n=4
Theoretical Conc. (ng/μL)	15	432	15	432
Ave. Value (ng/μL)	17.49	438.87	16.81	432.34
CV (%)	5.3	5.8	3.9	5.7
Relative bias (%)	16.6	1.6	12.1	0.1

7.2.2 Microplate Washer

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

Table 10. Instrument suitability results - Microplate Washer

Mode	Automatic washing	
QCs	Sample (Low) n=4	Sample (High) n=4
Theoretical Conc. (ng/μL)	15	432
Ave. Value (ng/μL)	14.85	400.63
CV (%)	3.6	3.1
Relative bias (%)	-1.0	-7.3

■ 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 BIOANALYTICAL METHOD VALIDATION

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

[6] Chinese pharmaceutical industry standard: YY/T1183-2010 Elisa reagent (kit)

Support & Contact

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