Validation Summary of

Vero HCP ELISA Kit (One-step ELISA)

■ INTRODUCTION

This report summarizes assay performance of SHENTEK® Vero 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® Vero HCP ELISA Kit (One-step ELISA), Product No. 1301309.
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 (%)
3	3.08	4.2	0.0
9	8.62	4.6	0.1
27	27.27	1.7	1.0
81	80.93	3.7	4.2
243	243.02	1.0	2.7
R^2	0.999		

2. Quantitation limit (QL)

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

Table2. Quantitation limit results

Theoretical Conc. (ng/mL)	CV (%)	Relative bias (%)
3 (n=10)	5.5	4.4
243 (n=10)	8.0	-2.9

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. 3 ng/mL), Low QC (Conc. 5 ng/mL), Medium QC (Conc. 50 ng/mL), High QC (Conc. 200 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	200	50	5	3
Ave. Value (ng/mL)	226.86	203.05	49.23	5.41	3.20
Recovery Rate (%)	93.4	101.5	98.5	108.1	106.7

5. Precision

5.1 Repeatability

Samples with three concentration points were tested 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)	200	50	5
Ave. Value (ng/mL)	193.43	47.69	4.76
CV (%)	4.1	2.8	5.0

5.2 Batch-to-batch precision

Two batches of the kit were tested in two separate assays to assess batch-to-batch precision, For ULOQ and LLOQ, the CV value was no more than25%, for high, medium and low samples, the CV value was no more than20%.

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)	243		200		50		5		3	
Ave. Value (ng/mL)	241.63	226.86	200.65	203.05	48.48	49.23	4.85	5.41	3.08	3.2
CV (%)	4.5		0.8		1.1		7.8		2.7	

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 average detection value of HCPs was less than the LLOQ and recovery rate was 75%-125%.

Table 6. Specificity results (for HCPs)

Host Cell Proteins	Ave. Value (ng/mL)	Spiked Conc. (ng/mL)	Recovery Rate (%)
Sf9 HCP	ND*	27	90.4
P.pastoris HCP	ND*	27	89.7
*E.*coli HCP	ND*	27	92.3

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

6.2 Selectivity (Matrix effect)

The recovery of Vero HCP spiked to 3 ng/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 (%)
20mM PB pH6.0	3	101.4
20mM PB pH7.0	3	118.4
20mM PB pH8.5	3	76.0

6.3 Antibody coverage

The HCP antibody coverage of Vero 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 65.1%-85.1%.

The antibody coverage obtained by IMBS-LC-MS method was 76.9% (Unique peptide≥2).

7. Robustness

7.1 Incubation condition

The suitable speed for incubation is at 500-600 rpm. Back-calculated concentration of calibration

standards in 500 rpm were evaluated by control condition indicated in user guide, the CV was no more than 15% and the relative bias was within±15%.

Table 8. Robustness results-Incubation speed

Incubation speed	500 rpm		
Theoretical Conc. (ng/mL)	Ave. Value (ng/mL) n=3	Relative bias (%)	CV (%)
243	199.9	-6.8	5.0
81	100.5	-4.3	3.0
27	49.4	-4.2	2.0
9	25.2	-1.4	0.9
3	10.1	-5.1	4.7

■ CONCLUSION

Parameters concluding linearity, range, QL, DL, specificity, 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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