Validation Summary of

MDCK HCP ELISA Kit (One-step ELISA)

■ INTRODUCTION

This report summarizes assay performance of SHENTEK® MDCK 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® MDCK HCP ELISA Kit (One-step ELISA), Product No. 1301308.
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 2-200 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 (%)
200	199.9	0.4	-0.1
100	100.5	1.5	0.5
50	49.4	3.5	-1.2
25	25.2	1.4	0.9
10	10.1	1.6	1.5
5	5.2	3.7	4.9
2	2.0	4.4	-2.0
R^2	0.99997		

2. Quantitation limit (QL)

The lower quantitation limit (LLOQ) of the assay is 2 ng/mL, and the upper quantitation limit (ULOQ) is 200 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 (%)
2 (n=10)	8.4	5.6
200 (n=10)	5.3	7.0

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 2 ng/mL.

4. Accuracy

The quality control samples (QCs) were prepared at 5 concentration levels within the calibration curve range: LLOQ (Conc. 2 ng/mL), Low QC (Conc. 6 ng/mL), Medium QC (Conc. 40 ng/mL), High QC (Conc. 150 ng/mL) and ULOQ (Conc. 200 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)	200	150	40	6	2
Ave. Value (ng/mL)	205.4	151.8	38.3	6.7	2.1
Recovery Rate (%)	102.7	101.2	95.8	110.8	106.0

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)	150	40	6
Ave. Value (ng/mL)	150.1	39.0	6.9
CV (%)	1.6	1.6	5.5

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 value was no more than 20%.

Table 5. Batch-to-batch precision results

QCs	Sample (High) n=10		Sample (Medium) n=10		Sample (Low) n=10	
Batch	1	2	1	2	1	2
Theoretical Conc. (ng/mL)	150		40		6	
Ave. Value (ng/mL)	150.1	146.7	39.0	39.8	6.9	6.0
CV (%)	5.1		3.6		9.3	

6. Specificity

6.1 Specificity for HCP

The HCPs of commonly used cell lines were prepared at 2 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 (%)
E.coli BL21 HCP	ND*	2	105.0
P.pastoris GS115 HCP	ND*	2	91.5
Sf9 HCP	ND*	2	103.2

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

6.2 Selectivity (Matrix effect)

The recovery of MDCK HCP spiked to 2 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 (%)
1×PBS, 0.075% Tween-20, 0.05% BSA, pH 6.5	2	103.4
1×PBS, 0.075% Tween-20, 0.05% BSA, pH 8.5	2	86.5
20 mM Tris-HCl, pH 7.4	2	93.0

6.3 Antibody Coverage

The HCP antibody coverage of MDCK 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 74.2%-89.7%.

The antibody coverage obtained by IMBS-LC-MS method was 70.1% (Unique peptide≥2).

7. Robustness

The assay is designed to conducted at 25℃±5℃. Sample incubation time may vary from 2.5 hours to 3.5 hours, the CV was no more than 20% and the relative bias was within±20%.

Table 8. 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	150	6	150	6	150
Ave. Value (ng/mL)	6.3	163.9	6.5	165.7	6.7	147.5
CV% (%)	0.9	4.2	1.2	13.0	7.5	4.8
Relative bias (%)	4.5	9.2	7.5	10.5	11.5	-1.7

Table 9. Robustness results-Sample incubation time

Time	2.5 h		3 h		3.5 h	
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	150	6	150	6	150
Ave. Value (ng/mL)	6.2	140.7	6.5	165.7	6.2	162.8
CV% (%)	7.3	1.3	1.2	13.0	3.9	2.0
Relative bias (%)	3.9	-6.2	7.5	10.5	3.3	8.5

■ 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)

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