Validation Summary of

CHO-K1 HCP ELISA Kit

(One-step ELISA)

■ INTRODUCTION

This report summarizes assay performance of SHENTEK® CHO-K1 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.

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® CHO-K1 HCP ELISA Kit (One-step ELISA) , Product No. 1301305-1
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 3-300 ng/mL, and $R^2 \geq 0.990$.The CV of the highest and lowest concentration points is not more than 25%, and the relative bias is within ±25%; CV of the remaining concentration points is not 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 Deviation(%)
300	300.36	8.5	0.1
150	149.67	6.8	-0.2
75	75.59	4.3	0.8
15	14.35	3.9	-4.3
6	6.26	5.5	4.3
3	3.11	3.8	3.8
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 300 ng/mL.The CV is not more than 25% and the relative bias is within 25%.

Table 2. Quantitative limit results

Theoretical Conc. (ng/mL)	Ave.value (ng/mL)	CV (%)	Relative Deviation (%)
3 (n=10)	3.66	5.3	22.2
300 (n=10)	284.07	3.7	-5.3

3. Accuracy

The quality control samples (QCs) were prepared at 5 concentration levels within the calibration curve range: The LLOQ（Conc. 3 ng/mL）, around two times of the LLOQ (Conc. 9 ng/mL), around the mid-range of the calibration curve (Medium QC Conc. 100 ng/mL), and at least above 75% of the ULOQ (High QC Conc. 240 ng/mL) and the ULOQ (Conc. 300 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)	300	240	100	9	3
Ave. Value (ng/mL)	282.62	244.14	102.22	9.00	3.68
Recovery Rate (%)	94.2	101.7	102.2	100.0	122.6

4. Precision

4.1 Repeatability

Precision were determined by analysing at least 10 replicates. CV values were not more than 20%.

Table 4. Repeatability results

QCs	Sample (High) n=10	Sample (Medium) n=10	Sample (Low) n=10
Theoretical Conc. (ng/mL)	240	100	9
Ave. Value (ng/mL)	238.55	102.65	9.14
CV (%)	6.1	2.6	4.7

4.2 Batch-to-batch precision

Two batches of the kit were tested in two separate assays to assess batch-to-batch precision.

Table 5. Batch-to-batch precision results

QCs	Sample (High) n=10		Sample (Medium) n=10		Sample (Low) n=10	
Theoretical Conc. (ng/mL)	240		100		9	
Batch	1	2	1	2	1	2
Ave. Value (ng/mL)	238.55	254.65	102.65	99.70	9.13	9.68
CV (%)	6.7		4.5		6.1	

5. Specificity

5.1 Specificity for HCP

The HCPs of commonly used cell lines were prepared in calibration standard diluent and assayed for cross-reactivity.

Table 6. Specificity results

Host Cell Proteins	Nominal Conc. (ng/mL)	Ave. Value (ng/mL)	Cross reactivity (%)
Sf9 HCP	3000 (10 × ULOQ)	< LLOQ	< 1
E. coli BL21 HCP	3000 (10 × ULOQ)	< LLOQ	< 1
HEK293T HCP	3000 (10 × ULOQ)	< LLOQ	< 1
P. pastoris HCP	3000 (10 × ULOQ)	Undet.	No cross-reactivity

5.2 Antibody coverage

The HCP antibody coverage of CHO-K1 HCP ELISA Kit (One-step ELISA) was evaluated by Immunomagnetic Bead Separation (IMBS) method combined with LC-MS (IMBS-LC-MS) analysis.

The antibody coverage obtained by IMBS-MS method was 94.6% (Unique peptide≥2).

6. Robustness

6.1 Incubation condition

The assay is designed to conducted at 25℃±5℃.The CV is not more than 20% and the relative bias is within 20%.

Table 7. Robustness results-Incubation temperature

Temperature	20℃		25℃		30℃	
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/mL)	9	240	9	240	9	240
Ave. Value (ng/mL)	9.62	220.48	9.50	253.15	10.00	243.09
CV(%)	7.8	7.0	3.6	3.5	6.6	2.6
Relative deviation%	6.9	-8.1	5.5	5.5	11.1	1.3

6.2 Termination condition

Plates read should be stable after termination but no more than 30 minute. The CV is not more than 20% and the relative bias is within 20%.

Table 8. Robustness results-Plate read

Time	+0 min		+10 min		+20 min		+30 min	
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	Sample (Low) n=4	Sample (High) n=4
Theoretical Conc. (ng/mL)	9	240	9	240	9	240	9	240
Ave. Value (ng/mL)	9.70	282.99	9.50	253.15	9.54	251.27	9.63	250.44
CV(%)	7.0	8.1	3.6	3.5	2.2	2.1	1.6	2.1
Relative deviation%	7.8	17.9	5.5	5.5	6.0	4.7	7.0	4.4

6.3 Reference wavelength

If equipped, reference wavelength (or long wavelength) should be 620-650 nm. If not, this step can be omitted. The CV is not more than 20% and the relative bias is within 20%.

Table 9. Robustness results-Reference wavelength

Ref.	None		620 nm		630 nm		640 nm		650 nm	
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	Sample (Low) n=4	Sample (High) n=4	Sample (Low) n=4	Sample (High) n=4
Theoretical Conc. (ng/mL)	9	240	9	240	9	240	9	240	9	240
Ave. Value (ng/mL)	10.00	259.23	10.02	258.62	10.01	258.64	10.01	258.70	10.00	258.71
CV(%)	2.7	5.0	3.1	5.0	3.0	5.0	3.0	5.0	3.0	5.0
Relative deviation%	11.1	8.0	11.3	7.8	11.2	7.8	11.3	7.8	11.1	7.8

6.4 Microplate Reader

The kit is applicable to but not limited to the following instruments.

Table 10. Instrument suitability results - Microplate Reader

Microplate Readers	Thermo Multiskan FC		MD Spectra Max ABS	
QCs	Sample (Low) n=4	Sample (High) n=4	Sample (Low) n=4	Sample (High) n=4
Theoretical Conc. (ng/mL)	9	240	9	240
Ave. Value (ng/mL)	9.50	253.15	10.02	258.62
CV(%)	3.6	3.5	3.1	5.0
Relative deviation(%)	5.5	5.5	11.3	7.8

■ CONCLUSION

Parameters including linearity, range, QL, specificity, precision, accuracy, and robustness were all evaluated and met the requirements.

■ REFERENCES

[1] USP <1225> Validation of Compendial Procedures

[2] USP <1103> Immunological Test Methods - Enzyme-Linked Immunosorbent Assay (ELISA)

[3] ICH M10: Bioanalytical Method Validation and Study Sample Analysis

[4] ICH Q2 (R2) Validation of Analytical Procedures

[5] ChP <9101> Guidance for Analytical Procedures Validation

[6] JP <G3-11-171> Enzyme-linked Immunosorbent Assay (ELISA)

Support & Contact

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