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 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

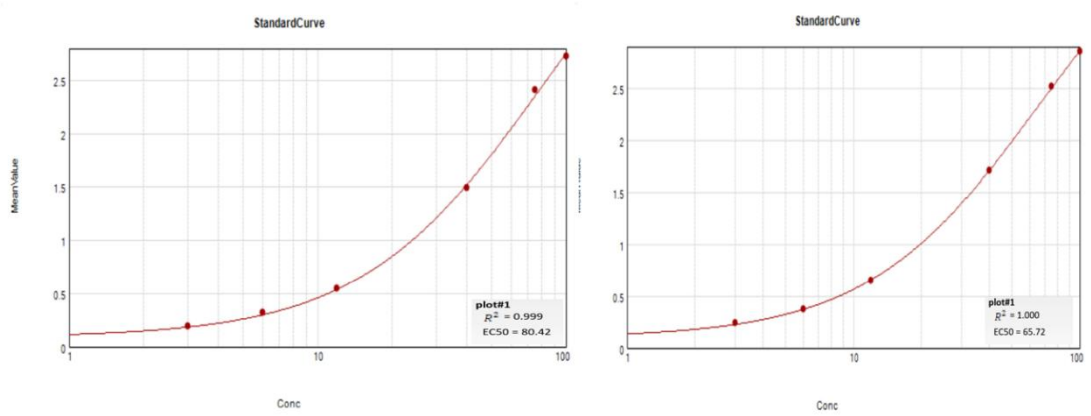
1. SHENTEK® CHO-K1 HCP ELISA Kit (One-step ELISA), Product No. 1301305.
2. The assay validation compliant with the pharmacopoeia requirement (e.g., USP<1132>,).
   Please refer to the reference for details.

■ RESULTS

1. Linearity and range

The assay range of the kit is 3-100 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%.

Figure 1. Linearity and range results



Analyst 1 Analyst 2

2. Quantitation limit (QL)

The lower quantitation limit (LLOQ) of the assay is 3 ng/mL. The CV is no more than 25% and the recovery is within 75%-125%.

Table1. Quantitation limit results

Sample	CHO-K1 HCP Calibration standards spiked Conc. (ng/mL)	Analyst	Parallel Test	Value (ng/mL)	Recovery (%)	CV (%)	
						n=3	n=6
1%BSA	3	Analyst1	1	3.203	107	1	4
			2	3.244	108		
			3	3.291	110		
		Analyst2	1	3.026	101	0	
			2	3.034	101		
			3	3.040	101		

3. Accuracy

The quality control samples (QCs) were prepared at 3 concentration levels within the calibration curve range: Low QC (Conc. 3 ng/mL), Medium QC (Conc.20 ng/mL), High QC (Conc.75 ng/mL).The recovery rate is 75%-125% for LLOQ（3ng/mL）, and 80%-120% for other samples.

Table 2. Accuracy results

Sample	CHO-K1 HCP Calibration standards spiked Conc. (ng/mL)	Parallel Test	Value (ng/mL)	Recovery (%)
1%BSA	75	1	75.370	100
		2	77.411	103
		3	74.885	100
	20	1	19.209	96
		2	18.660	93
		3	19.203	96
	3	1	3.203	107
		2	3.244	108
		3	3.291	110

4. Precision

4.1 Repeatability

Samples were tested for 6 times respectively, CV value was no more than 20%.

Table 3. Repeatability results

Sample	CHO-K1 HCP Calibration standards spiked Conc. (ng/mL)	Parallel Test	Value (ng/mL)	Ave. Value (ng/mL)	CV (%)
					n=6
1%BSA	20	1	19.209	19.124	1
		2	18.660		
		3	19.203		
		4	19.248		
		5	19.043		
		6	19.382		

4.2 Intermediate precision

Samples were tested by 2 analysts in 2 independent experiments, CV value was no more than 20%.

Table 4. Intermediate precision results

Sample	CHO-K1 HCP Calibration standards spiked Conc. (ng/mL)	Analyst	Parallel Test	Value (ng/mL)	Ave. Value (ng/mL)	CV (%) n=12
1%BSA	20	Analyst1	1	19.209	19.124	2
			2	18.660		
			3	19.203		
			4	19.248		
			5	19.043		
			6	19.382		
		Analyst2	1	18.365	18.541	
			2	18.664		
			3	18.736		
			4	18.639		
			5	18.628		
			6	18.213		

5. Specificity

5.1 Specificity for HCP

The HCPs of commonly used cell lines were prepared at 1000 ng/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 75%-125%.

Table 5. Specificity results

Host Cell Proteins	Ave. Value (ng/mL)	Spiked Conc.(ng/mL)	Recovery (%)
Sf9 HCP	/	3	101.5
293T HCP	/		101.1
E.*coli* HCP	/		103.6
MDCK HCP	0.40		116.1
P.pastoris GS115 HCP	/		84.7
P.pastoris X33 HCP	/		92.3
Vero HCP	0.57		111.8

5.2 Selectivity (Matrix effect)

The samples with pH between 5.5 and 7.5 showed no interference effect to the assay. The recovery rate was 75%-125%.

Table 6. Matrix interference results

sample matrix	dilution ratio	Spiked Conc.(ng/mL)	Recovery (%)
His buffer, 8%(w/v) sucrose, 0.02%(w/v) PS80, pH≈5.5	2	20	80-120
PBS buffer, 0.02% (w/v) PS80, pH≈7.5	20		

Note: The dilution of the matrix solution is consistent with the minimum dilution of the sample.

6. Robustness

6.1 Freeze-thaw stability

CHO-K1 HCP Calibration standards and Anti-CHO:HRP (250×) can be used no more than 3 freeze-thaw cycles and the performance of the kit is not affected.

6.2 Storage stability

The HCP Calibration standards and Anti-CHO:HRP (250×) in the kit were stably stored at ≤-18℃ for 24 months, and the performance of the kit is not affected.

■ 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

Support & Contact

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