Validation Summary of

P. pastoris HCP ELISA Kit (One-step ELISA)

■ INTRODUCTION

This report summarizes assay performance of SHENTEK® *P. pastoris* 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 kit is 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, selectivity, 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® *P. pastoris* HCP ELISA Kit (One-step ELISA), Product No. 1301313.
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 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 bias (%)
200	199.40	6.0	-0.3
100	101.60	6.3	1.6
50	49.02	1.8	-2.0
20	20.04	2.1	0.2
10	10.35	2.2	3.5
2	1.85	6.4	-7.5
R^2	4-PL, 0.999		

2. Quantitation limit (QL)

The lower quantitative limit (LLOQ) of the assay is 2 ng/mL, and the upper quantitative limit (ULOQ) is 200 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)	CV (%)	Relative bias (%)
2 (n=10)	9.7	3.5
200 (n=10)	6.0	4.6

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 0.5 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. 4 ng/mL), Medium QC (Conc. 80 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	80	4	2
Ave. value (ng/mL)	208.94	154.42	78.15	4.41	2.05
Recovery rate (%)	104.5	102.9	97.7	110.2	102.5

5. Precision

5.1 Repeatability

Samples with three concentration points were tested 10 times respectively, 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)	150	80	4
Ave. value (ng/mL)	159.59	78.85	4.44
CV (%)	6.1	3.0	3.9

5.2 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)	150		80		4	
Batch	1	2	1	2	1	2
Ave. value (ng/mL)	155.76	159.59	87.49	78.85	4.04	4.44
CV (%)	8.0		6.6		7.4	

6. Specificity

6.1 Specificity for HCP

The HCPs of commonly used cell lines were prepared at 1 μg/mL in calibration standard diluent and assayed for cross-reactivity.

Table 6. Specificity results

Host Cell Proteins	Ave. value (ng/mL)	Spiked Conc. (ng/mL)	Recovery rate (%)
Sf9 HCP	< LLOQ	2	81.8
CHO HCP	< LLOQ	2	76.3
E. coli BL21 HCP	< LLOQ	2	91.2
293T HCP	< LLOQ	2	80.7

6.2 Selectivity (Matrix effect)

The recovery of *P. pastoris* HCP spiked to 2 ng/mL (LLOQ) in commonly used matrices was evaluated. The tested matrices showed no interference to the assay.

Table 7. Selectivity results

Sample matrix	Spiked Conc. (ng/mL)	Recovery rate (%)
1×TBST, 1%BSA, pH 6.0	2	88.3
1×TBST, 1%BSA, pH 8.0	2	77.8
1×PBST, 0.25%BSA, pH 7.4	2	91.5

6.3 Antibody coverage

The HCP antibody coverage of *P. pastoris* HCP ELISA Kit (One-step ELISA) was evaluated by Immunomagnetic Bead Separation (IMBS) method combined with 2D-PAGE (IMBS-2D) or LC-MS (IMBS-LC-MS) analysis.

The antibody coverage obtained by IMBS-2D method was 72.1%-96.0%.

The antibody coverage obtained by IMBS-LC-MS method was 99.2%（Unique Peptide ≥2）.

7. Robustness

7.1 Incubation condition

The assay is designed to conducted at 25℃±5℃, Sample incubation time may vary from 2.5 hours to 3.5 hours. The suitable speed for incubation is at 400-600 rpm.

Table 8. 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)	4	150	4	150	4	150
Ave. Value (ng/mL)	4.09	141.96	4.05	167.30	3.97	155.46
CV (%)	3.7	3.6	4.5	10.3	7.9	18.0
Relative bias (%)	2.3	-5.4	1.2	11.5	-0.8	3.6

Table 9. Robustness results-Incubation time

Time	2.5 h		3 h		3.5 h	
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)	4	150	4	150	4	150
Ave. value (ng/mL)	4.06	158.70	3.99	155.42	4.26	151.34
CV (%)	5.8	9.4	0.6	6.0	7.5	3.1
Relative bias (%)	1.5	5.8	-0.3	3.6	6.6	0.9

Table 10. Robustness results-Incubation speed

Speed	400 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/mL)	4	150	4	150	4	150
Ave. value (ng/mL)	4.24	128.47	3.87	146.37	4.29	151.47
CV (%)	2.4	10.7	12.2	7.8	3.7	3.0
Relative bias (%)	6.0	-14.4	-3.2	-2.4	7.4	1.0

7.2 Instrument Suitability

7.2.1 Microplate Reader

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

Table 11. 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)	4	150	4	150
Ave. value (ng/mL)	3.99	155.42	3.79	145.90
CV (%)	0.6	6.0	2.5	1.8
Relative bias (%)	-0.3	3.6	-5.3	-2.7

7.2.2 Automatics System

The kit is suitable for automatic analytical systems.

Table 12. Instrument suitability results - Automatics System

Mode	SHENTEK® automatic analytical ELISA system	
QCs	Sample (Low) n=4	Sample (High) n=4
Theoretical Conc.(ng/mL)	4	150
Ave. value (ng/mL)	3.92	153.67
CV (%)	7.7	8.1
Relative bias (%)	-2.1	2.4

■ CONCLUSION

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

■ REFERENCES

[1] USP <1132> Residual Host Cell Protein Measurement in Biopharmaceuticals

[2] USP <1225> Validation of Compendial Procedures

[3] EP <2.6.34> Host-Cell Protein Assays

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

[5] ChP <9012> Guidance for Method Validation of Quantitative Analysis of BiologicalSamples

[6] JP <G3-9-172> Host Cell Protein Assay

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