Validation Summary of

Human IL-6 ELISA Kit

■ INTRODUCTION

This report summarizes assay performance of SHENTEK® Human IL-6 ELISA Kit. 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, selectivity, 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® Human IL-6 ELISA Kit, Product No. 1402431
2. The production of the kit is compliant with the requirements of ISO13485.
3. The assay validation compliant with the pharmacopoeia requirement (e.g., ChP<9012>, USP<1103>). Please refer to the reference for details.

■ RESULTS

1. Linearity and Range

The assay range of the kit is 2-512 pg/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. (pg/mL)	Ave.Value (pg/mL)	CV(%)	Relative bias(%)
2	1.91	3.5	-4.4
8	7.90	1.3	-1.3
16	15.80	1.0	-1.2
64	64.28	0.8	0.4
128	127.8	0.9	-0.1
512	512.06	2.8	0.0
R^2	4-PL, 0.999		

2. Quantitation limit (QL)

The lower quantitative limit (LLOQ) of the assay is 2 pg/mL, and the upper quantitative limit (ULOQ) is 512 pg/mL.The CV is not more than 25% and the relative bias is within ±25%.

Table2. Quantitative limit results

Theoretical Conc. (pg/mL)	Ave.Value (pg/mL)	CV(%)	Relative bias(%)
2 (n=10)	2.01	4.6	0.6
512 (n=10)	533.41	2.5	4.2

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 was 0.7 pg/mL.

4. Accuracy

The quality control samples (QCs) were prepared at 5 concentration levels within the calibration curve range:LLOQ（Conc. 2 pg/mL）, Low QC (Conc. 6 pg/mL), Medium QC (Conc. 200 pg/mL), High QC (Conc. 384 pg/mL) and ULOQ (Conc. 512 pg/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. (pg/mL)	512	384	200	6	2
Ave. Value (pg/mL)	538.13	396.87	219.68	5.78	1.96
Recovery Rate (%)	105.1	103.4	109.8	96.3	97.9

5. Precision

5.1 Repeatability

Samples with three concentration points were tested for 10 times respectively. The CV value is not more than 20%.

Table 4. Repeatability results

QCs	Sample (High)	Sample (Medium)	Sample (Low)
Theoretical Conc. (pg/mL)	384	200	6
Ave. Value (pg/mL)	392.84	214.72	5.75
CV(%)	2.5	2.4	6.7

6. Specificity

No cross reactivity was observed with the following cytokines by prepared at 1 ng/mL in calibration standard diluent and assayed using ELISA on a weight-to-weight bias. The mean value of the detection was less than the LLOQ and recovery rate was 80%-120%.

Table 5. Specificity results

Cytokines	Ave. Value (pg/mL)	Recovery rate (%)
Human IL-2	< LLOQ	96.7
Human IL-10	< LLOQ	105.3
Human IL-12	< LLOQ	94.9
Human IFN-γ	< LLOQ	103.1
Human IFN-β	< LLOQ	98.3
Human IL-6Rα	< LLOQ	97.5
Rat IL-6	< LLOQ	98.5
Mouse IL-6	< LLOQ	104.6

7. Robustness

7.1 Incubation condition

The assay is designed to conducted at 25℃±5℃. The kit is suitable for incubating on a microplate thermo shaker for 1 hours, as well as in a stationary incubator for 2 hours.The CV is not more than 20% and the relative bias is within ±20%.

Table 6. 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. (pg/mL)	6	384	6	384	6	384
Ave. Value (pg/mL)	5.84	419.77	6.23	445.04	5.91	413.32
CV(%)	3.3	5.6	6.8	5.3	3.3	5.4
Relative bias(%)	-2.6	9.3	3.8	15.9	-1.5	7.6

Table 7. Robustness results-Incubation condition

Incubation condition	Thermo shaker（1 hour）		Incubator（2 hours）	
QCs	Sample (Low) (n=3)	Sample(High) (n=3)	Sample (Low) (n=3)	Sample(High) (n=3)
Theoretical Conc. (pg/mL)	6	384	6	384
Ave. Value (pg/mL)	6.23	445.04	5.97	388.18
CV(%)	6.8	5.3	5.0	4.5
Relative bias(%)	3.8	15.9	-0.5	1.1

7.2 Instrument Suitability

7.2.1 Microplate Reader

The kit is applicable to but not limited to the following instruments. The CV is not more than 20% and the relative bias is within ±20%.

Table 8. Instrument suitability results - Microplate Reader

Microplate Readers	Thermo Multiskan FC		MD Spectra Max ABS	
QCs	Sample (Low) (n=3)	Sample (High) (n=3)	Sample (Low) (n=3)	Sample (High) (n=3)
Theoretical Conc. (pg/mL)	6	384	6	384
Ave. Value (pg/mL)	6.23	445.04	6.40	453.94
CV(%)	6.8	5.3	7.2	3.9
Relative bias(%)	3.8	15.9	6.6	18.2

7.2.2 Automatics System

The kit is suitable for automatic analytical ELISA systems.The CV is not more than 20% and the relative bias is within ±20%.

Table 9. Instrument suitability results - Automatics System

Mode	Manual Control		SHENTEK® automatic analytical ELISA system	
QCs	Sample(Low) (n=3)	Sample(High) (n=3)	Sample(Low) (n=3)	Sample(High) (n=3)
Theoretical Conc. (pg/mL)	6	384	6	384
Ave. Value (pg/mL)	6.39	431.79	6.17	385.52
CV(%)	9.9	1.1	13.2	3.7
Relative bias(%)	6.4	12.4	2.8	0.4

■ CONCLUSION

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

■ REFERENCES

[1] ICH Q2 (R2) ： VALIDATION OF ANALYTICAL PROCEDURES

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

[3] USP <1225> VALIDATION OF COMPENDIAL PROCEDURES

[4] ChP <9012> Guidance for method validation of quantitative analysis of biological samples

[5] JP <G1-1-130> Validation of Analytical Procedures

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

Support & Contact

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