# Validation Summary of

# CHO HCP ELISA Kit (One-step ELISA)

## ■ INTRODUCTION

This report summarizes assay performance of SHENTEK® CHO 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® CHO HCP ELISA Kit (One-step ELISA), Product No. 1301304-1.
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 1-128 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%.

Table1. Linearity and range results

| Theoretical Conc. (ng/mL) | Test 1 | | | Test 2 | | | Test 3 | | |
|---|---|---|---|---|---|---|---|---|---|
| | Ave.value (ng/mL) | CV (%) | Relative bias (%) | Ave.value (ng/mL) | CV (%) | Relative bias (%) | Ave.value (ng/mL) | CV (%) | Relative bias (%) |
| 1 | 1.08 | 11.8 | 7.8 | 1.06 | 5.8 | 6.2 | 0.99 | 5.5 | -0.8 |
| 2 | 1.95 | 6.2 | -2.7 | 2.03 | 8.2 | 1.4 | 1.94 | 5.7 | -3.1 |
| 4 | 3.94 | 3.2 | -1.6 | 3.84 | 2.4 | -4.0 | 4.10 | 3.5 | 2.5 |
| 16 | 16.06 | 4.5 | 0.4 | 16.10 | 1.7 | 0.6 | 15.96 | 4.1 | -0.3 |
| 64 | 63.97 | 1.8 | 0.0 | 63.95 | 1.7 | -0.1 | 64.07 | 6.6 | 0.1 |
| 128 | 128.02 | 0.6 | 0.0 | 128.08 | 4.6 | 0.1 | 128.04 | 3.8 | 0.0 |
| $R^2$ | 1.00000 | | | 0.99999 | | | 1.00000 | | |

### 2. Quantitation limit (QL)

The lower quantitation limit (LLOQ) of the assay is 1 ng/mL, and the upper quantitation limit(ULOQ) is 128 ng/mL. The CV is no more than25% and the relative bias is within±25%.

Table2. Quantitation limit results

| Theoretical Conc. (ng/mL) | Ave. Value (ng/mL) | CV(%) | Relative bias (%) |
|---|---|---|---|
| 1 (n=10) | 0.94 | 8.8 | -6.3 |
| 128 (n=10) | 125.55 | 2.7 | -1.9 |

### 3. Detection limit (DL)

The detection limit was defined as the detection concentration corresponding to the average value of the blank +2SD, and the detection limit of the kit is 0.19 ng/mL.

### 4. Accuracy

The quality control samples (QCs) were prepared at 5 concentration levels within the calibration curve range: LLOQ (Conc. 1 ng/mL), low QC (Conc. 3 ng/mL), medium QC (Conc. 50 ng/mL),

high QC (Conc. 100 ng/mL) and ULOQ (Conc. 128 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) | 128 | 100 | 50 | 3 | 1 |
| Ave. Value (ng/mL) | 127.42 | 99.04 | 52.38 | 2.86 | 0.93 |
| Recovery Rate (%) | 99.5 | 99.0 | 104.8 | 95.3 | 93.5 |

## 5. Precision

5.1 Repeatability

Samples with three concentration points were tested for 10 times respectively, CV values were no more than20%.

Table 4. Repeatability results

| QCs | Sample (high) | Sample (medium) | Sample (low) |
|---|---|---|---|
| Theoretical Conc. (ng/mL) | 100 | 50 | 3 |
| Ave. Value (ng/mL) | 98.06 | 51.22 | 2.93 |
| CV (%) | 3.5 | 3.6 | 5.6 |

5.2 Intermediate precision

Samples at five concentration points were tested by 2 technicians in 3 independent experiments. For ULOQ and LLOQ, the CV value was no more than25%, for high, medium and low samples, the CV value was no more than20%.

Table 5. Intermediate precision results

| QCs | Sample (ULOQ) n=30 | Sample (high) n=30 | Sample (medium) n=30 | Sample (low) n=30 | Sample (LLOQ) n=30 |
|---|---|---|---|---|---|
| Theoretical Conc. (ng/mL) | 128 | 100 | 50 | 3 | 1 |
| Ave. Value (ng/mL) | 113.04 | 91.49 | 48.52 | 2.91 | 1.00 |
| CV (%) | 7.5 | 8.6 | 6.5 | 7.8 | 14.6 |

## 6. Specificity

6.1 Specificity for HCP

The HCPs of commonly used cell lines were prepared at 1280 ng/mL in calibration standard diluent and assayed for cross-reactivity. The average detection value of HCPs was no more thanthe LLOQ and recovery rate was 75%-125%.

Table 6. Specificity results

| Host Cell Proteins | Ave. Value (ng/mL) | Spiked conc. (ng/mL) | Recovery Rate (%) |
|---|---|---|---|
| MDCK HCP | 0.02 | 1 | 79.8 |
| | | 128 | 88.9 |
| Vero HCP | 0.30 | 1 | 89.8 |
| | | 128 | 83.6 |
| 293T HCP | 0.14 | 1 | 83.9 |
| | | 128 | 87.6 |
| *P.pastoris* GS115 HCP | 0.10 | 1 | 90.7 |
| | | 128 | 93.8 |
| *E.coli* BL21 HCP | 0.22 | 1 | 76.2 |
| | | 128 | 95.3 |
| Sf9 HCP | 0.10 | 1 | 98.9 |
| | | 128 | 99.9 |
| *P.pastoris* X33 HCP | 0.03 | 1 | 106.0 |
| | | 128 | 112.7 |

6.2 Selectivity (Matrix effect)

The samples with pH between 6.5 and 8.5 showed no interference effect to the assay. The recovery rate was 75%-125%.

Table 7. Matrix interference results

| Spiked sample matrix | Spiked conc. (ng/mL) | Recovery Rate (%) |
|---|---|---|
| 20mM PB pH 6.5 | 1 | 102.4 |
| 20mM PB pH 7.0 | 1 | 102.2 |
| 20mM PB pH 8.5 | 1 | 79.4 |

6.3 Antibody coverage

The HCP antibody coverage of CHO 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 71.0%-82.6%.

The antibody coverage obtained by IMBS-LC-MS method was 86.8% (Unique peptide≥2).

## 7. Robustness

7.1 Incubation condition

The assay is designed to conducted at 25℃±3℃. For internal quality control , the CV was no more than20% and the relative bias was within ±20%. For ULOQ and LLOQ, the CV was no more than25% and the relative bias was within ±25%.

Table 8. Robustness results-Incubation condition

| Temperature | 22℃ | | | 28℃ | | |
|---|---|---|---|---|---|---|
| QCs | ULOQ (n=3) | LLOQ (n=3) | internal QC (n=2) | ULOQ (n=3) | LLOQ (n=3) | internal QC (n=2) |
| Theoretical Conc. (ng/μL) | 128 | 1 | 27.4 | 128 | 1 | 27.4 |
| Ave. Value (ng/μL) | 126.73 | 1.04 | 27.60 | 113.14 | 1.22 | 31.25 |
| CV (%) | 5.3 | 3.2 | 0.9 | 12.3 | 5.7 | 9.3 |
| Relative bias (%) | -1.0 | 4.3 | 0.7 | -11.6 | 22.2 | 14.0 |

## ■ 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)

## Support & Contact

SHENTEK

Huzhou Shenke Biotechnology Co., Ltd.
www.shentekbio.com
Address: 8th Floor, 6B Building, No.1366 Hongfeng Road, Huzhou313000, Zhejiang Province, China
E-mail: info@shentekbio.com
Phone: +1 (908) 822-3199 / (+86) 400-878-2189