Validation Summary of

ResHCD™ *Pichia Pastoris* DNA Quantitation Kit

■ INTRODUCTION

This report summarizes the analytical performance of ResHCD™ *Pichia Pastoris* DNA Quantitation Kit. The data presented herein are intended for reference purposes only. To demonstrate suitability for a specific intended purpose, appropriate validation or qualification study should be conducted with actual biological samples.

Parameters that may be evaluated for method validation are linearity, range, quantitation limit (QL), detection limit (DL), specificity, precision, accuracy, and robustness, etc.

The application of this report to actual biological samples should be assessed on a case-by-case basis. Users are encouraged to perform sample-specific suitability test, such as verification of QL and specificity and where necessary, additional validation activities to ensure compliance with relevant regulatory requirements.

The product described herein is intended for research use only and is not for use in diagnostic procedures or for therapeutic purposes in humans or animals.

■ MATERIALS & METHODS

1. Materials

ResHCD™ *Pichia Pastoris* DNA Quantitation Kit, Product No. 1101122.

SHENTEK® Residual Host Cell DNA Sample Preparation Kit (Fast), Product No. 1104201.

2. Methods

All reagents, materials, and standard operation procedures (SOPs) used in this study were developed and/or manufactured by Huzhou Shenke Biotechnology Co., Ltd.

The manufacturing processes for the kits are conducted in accordance with the quality management system requirements of ISO 13485.

■ RESULTS

1. Linearity and range

A DNA Control was serially diluted to six concentration levels and tested in triplicate. The study was repeated across three independent experiments.

The assay demonstrated a linear response over the range of 3.00E+02 to 1.00E-02 pg/μL (equivalent to 3.00E+03 to 1.00E-01 pg/reaction).

- Standard curve performance:
  - ✧ Test 1: $R^2 = 0.99886$; Efficiency (E) = 98.1%
  - ✧ Test 2: $R^2 = 0.99950$; E = 103.5%
  - ✧ Test 3: $R^2 = 0.99946$; E = 97.9%
- Acceptance criteria:
  - ✧ $R^2 \geq 0.990$
  - ✧ $90\% \leq E \leq 110\%$

All concentration levels met the predefined acceptance criteria, confirming acceptable linearity and analytical range.

2. Quantitation limit (QL)

The quantitation limit (QL) of the assay was established at 1.00E-02 pg/μL(1.00E-01 pg/reaction).

- Mean measured value: 9.43E-03 pg/μL
- Coefficient of variation (CV): 4.5%
- Relative bias: -5.7%
- Acceptance criteria:
  - ✧ CV ≤ 20%
  - ✧ Relative bias within ±20%

The QL met all acceptance criteria.

3. Detection limit (DL)

The detection limit (DL) was determined to be 1.00E-03 pg/μL (1.00E-02 pg/reaction).

- Detection rate: 100%
- Acceptance criterion:
  - ✧ Detection rate ≥ 95%

The DL met the predefined acceptance criterion.

4. Specificity

Potential interference from genomic DNA derived from common host engineering cells was evaluated, including CHO, *E. coli* and Vero . Each was tested at a concentration of 300 pg/μL (3 ng/reaction).

- CHO: Not detected
- *E. coli*: Not detected
- Vero: Not detected

All measured values were below the assay detection limit.

- Acceptance criterion:
  - ✧ Mean detected value below the assay DL

The assay demonstrated high specificity with no detectable cross-reactivity.

5. Repeatability

Repeatability was assessed using samples at three concentration levels. Each sample was independently prepared and analyzed.

- Observed CV values ranged from 4.5% to 23.2%
- Acceptance criterion:
  - ✧ CV ≤ 30%

All results met the acceptance criterion, indicating good repeatability.

6. Intermediate precision and Accuracy

Intermediate precision and accuracy were evaluated using samples at high, medium, and low concentration levels. Samples were prepared using the SHENTEK® Residual Host Cell DNA Sample Preparation Kit (Fast) and analyzed by three different operators, with each condition tested in triplicate.

- CV range: 3.8% – 17.9%
- Recovery range: 93.8% – 107.0%
- Acceptance criteria:
  - ✧ CV ≤ 30%
  - ✧ Recovery between 50% and 150%

All results met the predefined criteria, demonstrating acceptable intermediate precision and accuracy.

7. Robustness

Robustness was evaluated across multiple qPCR platforms, including SHENTEK-96H, ABI 7500, and LightCycler® 480 II.

- R^2 range: 0.99743 – 0.99946
- Efficiency range: 94.2% – 104.5%
- CV range: 4.5% – 17.8%
- Relative bias range: -5.7% – 15.3%
- Acceptance criteria:
  - ✧ $R^2 \geq 0.990$; $90\% \leq E \leq 110\%$
  - ✧ CV ≤ 20%; Relative bias within ±20%

All evaluated instruments met the acceptance criteria, confirming the robustness of the assay across platforms.

■ CONCLUSION

The ResHCD™ *Pichia Pastoris* DNA Quantitation Kit was systematically evaluated for key analytical performance characteristics, including linearity, range, quantitation limit, detection limit, specificity, precision, accuracy, and robustness.

All assessed parameters met the predefined acceptance criteria, demonstrating that the assay provides reliable, accurate, and reproducible quantitation of residual *Pichia Pastoris* host cell DNA under the tested conditions.

■ REFERENCES

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

[2] USP <509> Residual DNA Testing

[3] USP <1225> Validation of Compendial Procedures

[4] Ph.Eur. <2.6.35> Quantification and Characterisation of host-cell DNA

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

[6] ChP <3407> Exogenous DNA Residue Assay

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

Support & Contact

SHENTEK

Huzhou Shenke Biotechnology Co., Ltd.
www.shentekbio.com
Address:8th Floor, 6B Building, No.1366 Hongfeng Road, Huzhou 313000, Zhejiang Province, China
E-mail: info@shentekbio.com
Phone: +1 (908) 822-3199 / (+86) 400-878-2189