Validation Summary of

Residual E.coli DNA Quantitation Kit (2G)

■ INTRODUCTION

This report summarizes assay performance of SHENTEK® Residual E.coli DNA Quantitation Kit (2G) with sample preparation using SHENTEK® Residual Host Cell DNA Sample Preparation Kit. Both kits are 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), specificity, precision, accuracy and robustness, etc.

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® Residual E.coli DNA Quantitation Kit (2G), Product No. 1101107-1.
2. SHENTEK® Residual Host Cell DNA Sample Preparation Kit, Product No. 1104191.
3. The production of the kit is compliant with the requirements of ISO13485, and the assay validation compliant with the pharmacopoeia requirement.

■ RESULTS

1. Linearity and range

The range of the kit is 3.00E+02-3.00E-02 pg/μL, R^2=0.99968, amplification efficiency is 97.5%. Both CV and relative bias of the highest and lowest concentration points are no more than 20%; both CV and relative bias of the remaining concentration points are no more than 15%.

Table 1. Linearity and range results

Theoretical Conc. (pg/μL)	3.00E+02	3.00E+01	3.00E+00	3.00E-01	3.00E-02
Ave.Value (pg/μL)	3.12E+02	2.99E+01	2.86E+00	2.89E-01	3.17E-02
CV (%)	6.6	3.5	4.1	7.6	1.3
Relative bias (%)	4.0	0.4	4.7	3.7	5.7
R^2	0.99968				
Amplification efficiency (%)	97.5				

2. Accuracy

Sample preparation and detection assay were carried out for samples with high, medium and low spiked concentrations, and recovery and CV were analyzed. For different samples, the recovery rate is 50%-150%, and CV is less than 30%.

Table 2. Accuracy results

Matrix	No.	Theoretical Conc. (pg/μL)	Ave.Value (pg/μL)	CV (%)	Recovery rate (%)	Acceptance Criteria	Conclusion
10 mM PB, 0.3 M NaCl, 10 mg/mL BSA (pH=6.0±0.5) solution	Unspiked sample	NA	5.36E-04	NA	NA	1. The recovery rate is 50%-150%. 2. CV≤30%.	NA
	Sample (low)	6.00E-02	5.76E-02	8.3	95.1		Pass
	Sample (medium)	3.00E-01	2.76E-01	3.0	91.8		Pass
	Sample (high)	3.00E+01	3.15E+01	8.3	105.1		Pass
50 mM NaAc/HAc, 50 mM NaCl, 10 mg/mL BSA (pH=5.0±0.5) solution	Unspiked sample	NA	Undet.	NA	NA		NA
	Sample (low)	6.00E-02	6.45E-02	3.0	107.4		Pass
	Sample (medium)	3.00E-01	2.87E-01	8.7	95.7		Pass
	Sample (high)	3.00E+01	2.85E+01	0.3	95.1		Pass
50 mM Tris-Cl, 10 mg/mL BSA (pH=8.5±0.5) solution	Unspiked sample	NA	1.30E-03	NA	NA		NA
	Sample (low)	6.00E-02	5.79E-02	16.5	94.4		Pass
	Sample (medium)	3.00E-01	2.81E-01	1.3	93.3		Pass
	Sample (high)	3.00E+01	2.81E+01	1.6	93.6		Pass
0.75 M $(NH_4)_2SO_4$, 50 mM Na_3PO_4, 10 mg/mL BSA (pH=6.0±0.5) solution	Unspiked sample	NA	4.38E-04	NA	NA		NA
	Sample (low)	6.00E-02	5.06E-02	9.7	83.6		Pass
	Sample (medium)	3.00E-01	2.71E-01	8.0	90.1		Pass
	Sample (high)	3.00E+01	2.84E+01	0.4	94.5		Pass
50 mM Citric acid /Sodium citrate, 0.2 M NaCl, 10 mg/mL BSA (pH=4.0±0.5)	Unspiked sample	NA	Undet.	NA	NA		NA
	Sample (low)	6.00E-02	5.74E-02	16.9	95.7		Pass
	Sample (medium)	3.00E-01	2.82E-01	6.5	94.1		Pass
	Sample (high)	3.00E+01	2.84E+01	1.1	94.7		Pass
50 mM Tris, 20 mM NaAc, 10 mg/mL BSA (pH=7.5±0.5) solution	Unspiked sample	NA	6.25E-04	NA	NA		NA
	Sample (low)	6.00E-02	4.93E-02	18.7	81.2		Pass
	Sample (medium)	3.00E-01	2.64E-01	3.5	87.8		Pass
	Sample (high)	3.00E+01	2.92E+01	5.6	97.4		Pass

3. Quantitation limit (QL)

The quantitation limit of the kit is 3.00E-02 pg/μL.

Table 3. Quantitation limit results

Theoretical Conc. (pg/μL)	3.00E-02
Ave. Value (pg/μL)	3.32E-02
CV (%)	11.6
Relative bias (%)	10.8
Acceptance Criteria	1. CV≤20%. 2. Relative bias≤20%.
Conclusion	Pass

4. Detection limit (DL)

The detection limit of the kit is 3.00E-03 pg/μL.

Table 4. Detection limit results

Theoretical Conc. (pg/μL)	3.00E-03
Detection rate (%)	95.8
Acceptance Criteria	Detection rate ≥ 95%.
Conclusion	Pass

5. Specificity

The interference of common engineering cell genomes to the kit was evaluated, which contained CHO, 293T, and *Pichia pastoris* The results showed that the detection mean of CHO, 293T, and *Pichia pastoris* genomic DNA of 3 ng were lower than the detection limit of the kit, so the above interference DNA did not interfere with the kit.

Table 5. Specificity results

gDNA	Ave. Value (pg/μL)	Acceptance Criteria	Conclusion
CHO	Undet	The mean value of the detection is less than DL.	Pass
293T	Undet		Pass
Pichia pastoris	9.44E-04		Pass

6. Precision

6.1 Repeatability

Samples with two concentration points were tested for 6 times respectively, and CV value was less than 30%.

Table 6. Repeatability results

Theoretical Conc.(pg/μL)	3.00E+01	3.00E-01
Ave. Value (pg/μL)	2.77E+01	3.12E-01
CV (%)	2.8	17.9
Acceptance Criteria	CV≤30%.	
Conclusion	Pass	Pass

6.2 Intermediate precision

Samples with three concentration points were tested by 2 technicians for total 3 individual results and CV values for all samples were less than 30%.

Table 7. Intermediate precision results

Theoretical Conc. (pg/μL)	3.00E+02	3.00E+00	6.00E-02
Ave. Value (pg/μL)	2.63E+02	2.67E+00	6.04E-02
CV (%)	2.2	6.4	16.5
Acceptance Criteria	CV≤30%.		
Conclusion	Pass	Pass	Pass

7. Robustness

7.1 Freeze-thaw stability

SHENTEK® Residual E.coli DNA Quantitation Kit (2G) has stable assay performance for at least 5 freeze-thaw cycles.

Table 8. Freeze-thaw stability results

Item	Parameters		Acceptance Criteria	Conclusion
Amplification efficiency (%)	93.3		1. R^2≥0.990, 83.3%≤E≤110%; 2. Both CV and Relative bias≤20%.	Pass
R^2	0.99972			
Accuracy (3.00E+02 pg/μL)	CV (%)	1.2		Pass
	Relative bias (%)	5.2		
Repeatability (3.00E-02 pg/μL)	CV (%)	13.9		Pass

7.2 Instrument suitability

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

Table 9. Instrument suitability results

Supplier	Model	Amplification efficiency (%)	R^2	Test Conc. (pg/μL)	CV (%)	Relative bias (%)	Conclusion
HZSKBio	SHENTEK-96S	93.1	0.99962	3.00E-02	19.6	4.3	Pass
Thermo	ABI 7500	94.9	0.999	3.00E-02	10.2	16.0	Pass
Bio-Rad	CFX-96	94.8	0.999	3.00E-02	18.8	17.3	Pass
Acceptance Criteria	1. R^2≥0.990、83.3%≤E≤110%; 2. CV and Relative bias≤20%.						

■ CONCLUSION

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

■ REFERENCES

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

[2] USP <509> RESIDUAL DNA TESTING

[3] USP <1225> VALIDATION OF COMPENDIAL PROCEDURES

[4] ChP <9012> Bioanalytical method validation guideline

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

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