

Validation Summary of Residual Vero DNA-154 Quantitation Kit

■ INTRODUCTION

This report summarizes assay performance of SHENTEK® Residual Vero DNA-154 Quantitation Kit with sample preparation using SHENTEK® Residual Host Cell DNA Sample Preparation Kit For Vaccines. 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 Vero DNA-154 Quantitation Kit, Product No. SK030227V100.
2. SHENTEK® Residual Host Cell DNA Sample Preparation Kit For Vaccines, Product No. SK030206DM50.
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-03 - 3.00E+01 pg/μL. $R^2=0.99898$, amplification efficiency is 92.5%.

Table 1. Linearity and range results

Theoretical Conc. (pg/μL)	3.00E+01	3.00E+00	3.00E-01	3.00E-02	3.00E-03	3.00E-04
Ave. Value (pg/μL)	3.09E+01	2.73E+00	2.64E-01	2.75E-02	3.03E-03	3.12E-04
CV (%)	4.7	0.4	0.9	2.5	10.2	87.0
Relative bias (%)	3.1	9.0	11.8	8.5	0.9	4.0
Acceptance Criteria	1. $R^2 \geq 0.990$, $83.3\% \leq E \leq 110\%$; 2. Both CV and relative bias of the highest and lowest concentration points are not more than 20%; 3. Both CV and relative bias of the remaining concentration points are not more than 15%.					
Conclusion	Pass	Pass	Pass	Pass	Pass	Fail

2. Accuracy

Sample preparation and detection assay were carried out for samples with high, medium and low spiked concentrations, and the recovery rate and CV were analyzed. For different samples, the recovery rate should be 50%-150%, and CV should be not more than 30%.

Table 2. Accuracy results

Sample group	Unspiked sample	Sample (high)	Sample (medium)	Sample (low)
Theoretical Conc. (pg /μL)	0.00E+00	3.00E+01	3.00E-01	6.00E-03
Ave. Value (pg/μL)	1.36E-04	2.94E+01	2.63E-01	6.05E-03
CV (%)	N/A	11.0	3.6	9.3
Recovery rate (%)	N/A	98.0	87.7	98.5
Acceptance Criteria	1. The recovery rate is 50%-150%; 2. $CV \leq 30\%$.			
Conclusion	NA	Pass	Pass	Pass

3. Quantitation limit (QL)

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

Table 3. Quantitation limit results

Theoretical Conc. (pg/μL)	3.00E-03
Ave. Value (pg/μL)	2.95E-03
CV (%)	9.0
Relative bias (%)	1.6
Acceptance Criteria	Both relative bias and CV are not more than 20%.
Conclusion	Pass

4. Detection limit (DL)

The detection limit of the kit is 3.00E-04 pg/μL.

Table 4. Detection limit results

Theoretical Conc. (pg/μL)	3.00E-04
Detection rate(%)	100
Acceptance Criteria	Detection rate ≥ 95%
Conclusion	Pass

5. Specificity

The interference of commonly used engineering cell or engineering bacteria genomes to the assay was evaluated, which contained CHO, *E. coli*, 293T, HEK293, NS0, MDCK and *Pichia pastoris*. The results showed that the mean values of detection assay of 3 ng genomic DNA were lower than the detection limit of the kit, so the cross-reaction of these off-target DNA yielded a negative result and demonstrated no interference with the assay.

Table 5. Specificity results

gDNA	Ave. value (pg/μL)	Acceptance Criteria	Conclusion
CHO	2.32E-04	The mean value of the detection is less than the detection limit.	No interference
<i>E.coli</i>	<1.000E-5		No interference
293T	<1.000E-5		No interference
HEK293	<1.000E-5		No interference
<i>Pichia pastoris</i>	2.57E-05		No interference
NS0	<1.000E-5		No interference
MDCK	1.15E-05		No interference

6. Precision

6.1 Repeatability

Samples with two concentration points were tested for 6 times respectively, and CV value was not more than 30%.

Table 6. Repeatability results

Theoretical Conc. (pg/μL)	Ave. Value (pg/μL)	CV (%)	Acceptance Criteria	Conclusion
3.00E+00	2.82E+00	7.0	CV≤30%.	Pass
3.00E-02	2.54E-02	6.2		Pass

6.2 Intermediate precision

Samples with three concentration points were tested by 2 technicians for total 3 individual results and CV value for all samples were not more than 30%.

Table 7. Intermediate precision results

Theoretical Conc. (pg/μL)	3.00E+01	3.00E-01	6.00E-03
Ave. Value (pg/μL)	2.50E+01	2.35E-01	5.13E-03
CV (%)	16.7	12.4	18.3
Acceptance Criteria	CV≤30%.		
Conclusion	Pass	Pass	Pass

7. Robustness

7.1 Freeze-thaw stability

SHENTEK® Residual Vero DNA-154 Quantitative Kit had 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 or $ R \geq 0.990$, $83.3\% \leq E \leq 110\%$; 2. Accuracy: CV and Relative bias $\leq 15\%$; 3. Repeatability: CV $\leq 15\%$. (for QL, CV and Relative bias $\leq 20\%$)	Pass
R^2 or $ R $	0.99987			Pass
Accuracy (3.00E+01 pg/ μ L)	CV(%)	3.0		Pass
	Relative bias (%)	0.6		Pass
Repeatability (3.00E-03 pg/ μ L)	CV(%)	11.4		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 or $ R $	Test Conc. (pg/ μ L)	CV (%)	Relative bias (%)	Conclusion
HZSKBIO	SHENTEK-96S	90.4	0.99952	$3.00E-03$	9.0	1.6	Pass
Thermo	7500	90.2	0.999505	$3.00E-03$	17.9	6.8	Pass
Bio-Rad	CFX-96	100.5	1.000	$3.00E-03$	15.4	10.8	Pass
Bioer	FQD-96A	93.0	0.999	$3.00E-03$	16.7	7.0	Pass
Roche	Light Cycler 480 II	92.6	0.9995	$3.00E-03$	17.3	13.0	Pass
Jena	qTOWER ³ G	92.0	0.99781	$3.00E-03$	12.2	5.7	Pass
Acceptance Criteria		1. R^2 or $ R \geq 0.990$, $83.3\% \leq E \leq 110\%$; 2. CV and Relative bias $\leq 20\%$.					

■ 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

The logo for SHENTEK, with the word in a bold, sans-serif font. The 'S' and 'H' are blue, while the 'E', 'N', 'T', 'E', and 'K' are green.

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