Validation Summary of

Residual Vero DNA Size Analysis Kit (2G)

■ INTRODUCTION

This report summarizes assay performance of SHENTEK® Residual Vero DNA Size Analysis Kit (2G) with sample preparation using SHENTEK® Residual 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), detection limit (DL), specificity, 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® Residual Vero DNA Size Analysis Kit (2G), Product No. 1103174.
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 4 detection assays is 3.00E-02-3.00E+02 pg/μL. and R^2 ≥0.990; the amplification efficiency is 83.3%-110% .

Table 1. Linearity and range results

DNA size	Range (pg/μL)	R^2	Amplification efficiency (%)	Acceptance Criteria	Conclusion
85 bp	3.00E-02-3.00E+02	0.99982	96.7	1.R^2≥0.990, 83.3%≤E≤110%; 2. For highest and lowest concentration: 85 bp, 134 bp and 229 bp assays, both Relative bias and CV are no more than 30%; 552 bp assay, both Relative bias and CV are no more than 40%. For the other concentrations: both Relative bias and CV are no more than 20%.	Pass
134 bp	3.00E-02-3.00E+02	0.99938	96.4		Pass
229 bp	3.00E-02-3.00E+02	0.99982	91.8		Pass
552 bp	3.00E-02-3.00E+02	0.99924	92.8		Pass

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 no more than 40%.

Table 2. Accuracy results

DNA Size	85 bp			134 bp			229 bp			552 bp		
Theoretical Conc. (pg/μL)	3.00E+02	3.00E+00	6.00E-02	3.00E+02	3.00E+00	6.00E-02	3.00E+02	3.00E+00	6.00E-02	3.00E+02	3.00E+00	6.00E-02
Ave. Value (pg/μL)	2.64E+02	2.58E+00	4.94E-02	2.36E+02	2.13E+00	4.68E-02	2.46E+02	2.98E+00	5.53E-02	2.65E+02	2.26E+00	6.05E-02
CV (%)	21.8	2.2	19.0	7.5	9.7	3.4	14.1	13.5	10.6	17.8	10.7	5.6
Recovery rate (%)	87.9	86.0	82.2	78.8	71.1	78.0	82.1	99.4	91.5	88.3	75.2	97.9
Acceptance Criteria	1. The recovery rate is 50%-150%. 2. CV≤40%											
Conclusion	Pass	Pass	Pass	Pass	Pass	Pass	Pass	Pass	Pass	Pass	Pass	Pass

3. Quantitation Limit (QL)

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

Table 3. Quantitation limit results

DNA Size	85 bp	134 bp	229 bp	552 bp
R^2	0.99984	0.99974	0.99934	0.99666
Amplification efficiency(%)	92.7	94.6	96.2	99.6
Theoretical Conc. (pg/μL)	3.00E-02	3.00E-02	3.00E-02	3.00E-02
Ave. Value (pg/μL)	2.90E-02	2.85E-02	3.03E-02	3.58E-02
CV (%)	7.1	10.3	14.4	6.8
Relative bias (%)	3.4	5.0	0.9	19.3
Acceptance Criteria	1.R^2≥0.990, 83.3%≤E≤110%; 2. Both CV and Relative bias are no more than 30% for 85 bp, 134bp and 229 bp; both CV and Relative bias are no more than 40% for 552 bp.			
Conclusion	Pass	Pass	Pass	Pass

4. Detection Limit (DL)

The detection limit of 85 bp detection assay is 6.00E-03 pg/μL, and the detection limit of 134 bp, 229 bp and 552 bp detection assays is 1.00E-03 pg/μL.

Table 4. Detection limit results

DNA Size	85 bp	134 bp	229 bp	552 bp
Theoretical Conc. (pg/μL)	6.00E-03	1.00E-03	1.00E-03	1.00E-03
Detection rate (%)	100	100	100	100
Acceptance Criteria	Detection rate≥95%.			
Conclusion	Pass	Pass	Pass	Pass

5. Specificity

The interference of CHO, *E. coli*, *Pichia pastoris*, and MDCK were evaluated. The results showed that the mean value of detection assay of the cell genomic DNA of 3 ng 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

DNA Size	gDNA	Ave. value (pg/μL)	Acceptance Criteria	Conclusion
85 bp	CHO	Undet.	The mean value of the detection is no more than the detection limit.	No interference
	E. coli	Undet.		No interference
	Pichia pastoris	Undet.		No interference
	MDCK	Undet.		No interference
134 bp	CHO	5.51E-04		No interference
	E. coli	Undet.		No interference
	Pichia pastoris	Undet.		No interference
	MDCK	Undet.		No interference
229 bp	CHO	3.26E-04		No interference
	E. coli	Undet.		No interference
	Pichia pastoris	3.53E-04		No interference
	MDCK	Undet.		No interference
552 bp	CHO	8.06E-04		No interference
	E. coli	5.07E-04		No interference
	Pichia pastoris	3.02E-04		No interference
	MDCK	3.98E-04		No interference

6. Precision

6.1 Repeatability

Samples at two concentration points were tested for 6 times respectively for each detection assay, and CV value was no more than 40%.

Table 6. Repeatability results

DNA Size	85 bp		134 bp		229 bp		552 bp	
Theoretical Conc. (pg/μL)	3.00E+01	3.00E-01	3.00E+01	3.00E-01	3.00E+01	3.00E-01	3.00E+01	3.00E-01
Ave. Value (pg/μL)	2.75E+01	2.92E-01	2.38E+01	2.66E-01	2.93E+01	2.56E-01	2.59E+01	2.35E-01
CV (%)	12.1	12.3	8.5	13.6	8.7	11.3	5.5	6.2
Acceptance Criteria	CV≤40%							
Conclusion	Pass	Pass	Pass	Pass	Pass	Pass	Pass	Pass

6.2 Intermediate precision

Each of Samples at three concentration points were tested in triplicate by 3 different technicians for each detection assay, and CV values for every sample were no more than 40%.

Table 7. Intermediate precision results

DNA Size	85 bp			134 bp			229 bp			552 bp		
Theoretical Conc. (pg/μL)	3.00E+02	3.00E+00	6.00E-02	3.00E+02	3.00E+00	6.00E-02	3.00E+02	3.00E+00	6.00E-02	3.00E+02	3.00E+00	6.00E-02
Ave. Value (pg/μL)	2.44E+02	2.39E+00	4.93E-02	2.08E+02	1.87E+00	4.16E-02	2.25E+02	2.38E+00	4.52E-02	2.75E+02	2.46E+00	5.44E-02
CV (%)	14.4	11.4	11.5	13.8	24.2	25.8	19.2	23.7	23.6	15.9	18.4	20.1
Acceptance Criteria	CV≤40%.											
Conclusion	Pass	Pass	Pass	Pass	Pass	Pass	Pass	Pass	Pass	Pass	Pass	Pass

7. Robustness

7.1 Freeze-thaw stability

SHENTEK® Residual Vero DNA Size Analysis Kit (2G) has stable assay performance for at least 5 freeze-thaw cycles.

Table 8. Freeze-thaw stability results

DNA Size		85 bp	134 bp	229 bp	552 bp
Amplification efficiency (%)		97.8	94.0	95.8	92.9
R^2		0.99974	0.99862	0.99924	0.99978
Accuracy (3.00E+02 pg/μL)	CV (%)	4.5	15.9	9.2	11.1
	Relative bias (%)	10.8	1.1	3.2	1.1
Repeatability (3.00E-02 pg/μL)	CV (%)	9.1	15.3	10.8	11.9
Conclusion		Pass	Pass	Pass	Pass
Acceptance Criteria	1. $R^2 \geq 0.990$,83.3%≤E≤110%; 2. Accuracy: Both CV and Relative bias are no more than 30% for 85 bp, 134 bp and 229 bp; both CV and Relative bias are no more than 40% for 552 bp. 3. Repeatability: CV is no more than 30% for 85 bp, 134 bp and 229 bp; CV is no more than 40% for 552 bp.				

7.2 Instrument suitability

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

Table 9. Instrument suitability results

Supplier	Model	DNA Size	Test Conc. (pg/μL)	Amplification efficiency(%)	R^2	CV(%)	Relative bias(%)	Conclusion
SHENTEK	SHENTEK-96S	85 bp	3.00E-02	92.7	0.99984	7.1	3.4	Pass
		134 bp	3.00E-02	94.6	0.99974	10.3	5.0	Pass
		229 bp	3.00E-02	96.2	0.99934	14.4	0.9	Pass
		552 bp	3.00E-02	99.6	0.99666	6.8	19.3	Pass
Thermo	ABI 7500	85 bp	3.00E-02	95.6	0.999540	10.9	5.2	Pass
		134 bp	3.00E-02	92.4	0.998173	14.6	17.9	Pass
		229 bp	3.00E-02	91.9	0.999212	9.3	16.2	Pass
		552 bp	3.00E-02	87.1	0.999012	13.6	18.1	Pass
Bio-Rad	CFX-96	85 bp	3.00E-02	105.0	1.000	12.2	0.6	Pass
		134 bp	3.00E-02	100.7	0.999	6.8	0.7	Pass
		229 bp	3.00E-02	100.2	0.999	6.4	3.2	Pass
		552 bp	3.00E-02	94.9	0.999	8.6	1.0	Pass
Roche	LightCycler 480	85 bp	3.00E-02	99.3	0.9996	12.6	1.2	Pass
		134 bp	3.00E-02	97.5	0.9991	10.1	16.1	Pass
		229 bp	3.00E-02	96.2	0.9990	11.3	1.1	Pass
		552 bp	3.00E-02	91.5	0.9983	11.3	6.6	Pass
Jena	qTOWER^3	85 bp	3.00E-02	86.0	0.99127	16.0	0.2	Pass
		134 bp	3.00E-02	84.0	0.99861	18.7	18.4	Pass
		229 bp	3.00E-02	87.0	0.99936	14.8	13.4	Pass
		552 bp	3.00E-02	93.0	0.99586	10.2	0.5	Pass
Acceptance Criteria	1. $R^2 \geq 0.990$,83.3%≤E≤110%; 2. Both CV and Relative bias are no more than 30% for 85bp, 134 bp and 229 bp; Both CV and Relative bias are no more than 40% for 552bp.							

■ CONCLUSION

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

■ REFERENCES

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[2] USP <509> RESIDUAL DNA TESTING

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