Validation Summary of

Deoxyribonuclease I (DNase I) ELISA Kit

■ INTRODUCTION

This report summarizes assay performance of SHENTEK® Deoxyribonuclease I (DNase I) ELISA Kit. 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), 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® DNase I ELISA Kit, Product No. 1402428
2. The production of the kit is compliant with the requirements of ISO13485.
3. The assay validation compliant with the pharmacopoeia requirement (*e.g.*, ICH Q2(R2)). Please refer to the reference for details.

■ RESULTS

1. Linearity and Range

The assay range of the kit is 0.5 - 32 ng/mL, and $R^2 \geq 0.990$.The CV of the highest and lowest concentration points is not more than 25%, and the relative bias is within ±25%; CV of the remaining concentration points is not more than 20%, and the relative bias is within ±20%.

Table 1. Linearity and range results

Theoretical Conc. (ng/mL)	Ave.value (ng/mL)	CV(%)	Relative bias(%)
32	1.49	8.8	0.4
16	1.19	5.7	0.8
8	0.88	4.1	-2.6
4	0.63	1.5	0.9
2	0.45	3.5	6.5
0.5	0.17	1.7	-17.3
R^2	4-PL, 0.999		

2. Quantitation Limit (QL)

The lower quantitative limit (LLOQ) of the assay is 0.5 ng/mL, and the upper quantitative limit (ULOQ) is 32 ng/mL.The CV is not more than 25% and the relative bias is within ±25%.

Table 2. Quantitative limit results

Theoretical Conc. (ng/mL)	Ave.value (ng/mL)	CV (%)	Relative bias (%)
0.5 (n=10)	0.45	4.2	-10.6
32 (n=10)	32.2	9.0	0.8

3. Detection Limit (DL)

The detection limit was defined as the detection concentration corresponding to the average value (n=20) of the blank +2SD, and the detection limit of the kit was within 0.1 ng/mL.

4. Accuracy

The quality control samples (QCs) were prepared at 5 concentration levels within the calibration curve range:LLOQ (Conc. 0.5 ng/mL), Low QC (Conc. 1.5 ng/mL), Medium QC (Conc. 13 ng/mL), High QC (Conc. 24 ng/mL) and ULOQ (Conc. 32 ng/mL).

The recovery 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)	32	24	13	1.5	0.5
Ave. value (ng/mL)	29.90	23.42	11.60	1.64	0.47
Recovery (%)	93.4	97.6	89.2	109.4	93.2

5. Precision

5.1 Repeatability

Samples with three concentration points were tested for 10 times respectively. The CV value is not more than 20%.

Table 4. Repeatability results

QCs	Sample (High)	Sample (Medium)	Sample (Low)
Theoretical Conc. (ng/mL)	24	13	1.5
Ave. value (ng/mL)	23.84	12.26	1.78
CV(%)	8.7	7.8	6.6

6. Specificity

6.1 Specificity for Interference Substances

No cross reactivity was observed with the following interference by prepared at 320 ng/mL in calibration standard diluent. The mean value of the detection is not more than the LLOQ and recovery is 80%-120%.

Table 5. Specificity results

Interference substances	Ave. value (ng/mL)	Spiked Conc. (ng/mL)	Recovery (%)
P. pastoris GS115 HCP	< LLOQ	24	83.5
		1.5	105.0
Vero HCP	< LLOQ	24	86.6
		1.5	99.5
293T HCP	< LLOQ	24	80.7
		1.5	95.2
CHO HCP	< LLOQ	24	93.9
		1.5	111.8
Sf9 HCP	< LLOQ	24	89.9
		1.5	102.7
E. coli BL21 HCP	< LLOQ	24	91.3
		1.5	109.0
T7 RNA Polymerase	< LLOQ	24	116.9
		1.5	122.3
RNase Inhibitor	< LLOQ	24	100.4
		1.5	114.9

6.2 Selectivity (Matrix effect)

The recovery of DNase I spiked to 0.5 ng/mL (LLOQ) and 32 ng/mL (ULOQ) in commonly used matrices was evaluated. The results showed recoveries within the acceptable range of 75% to 125%, and the matrices showed no interference with the assay.

Table 6. Selectivity results

Sample matrix	Spiked Conc. (ng/mL)	Recovery (%)
1×PBS, 0.075% Tween-20, 0.5% BSA, pH 8.0	0.5	104.5
	32	79.8
1×PBS, 0.075% Tween-20, 0.5% BSA, pH 6.5	0.5	75.9
	32	97.9

7. Robustness

7.1 Incubation Condition

The assay is designed to conducted at 25℃±3℃. The suitable speed for sample incubation is at 400-600 rpm. The CV is not more than 20% and the relative bias is within ±20%.

Table 7. Robustness results-Incubation condition

Temperature	22℃		25℃		28℃	
Sample incubation speed	400rpm		600rpm		600rpm	
QCs	Sample (Low) (n=4)	Sample (High) (n=4)	Sample (Low) (n=4)	Sample (High) (n=4)	Sample (Low) (n=4)	Sample (High) (n=4)
Theoretical Conc.. (ng/mL)	1.5	24	1.5	24	1.5	24
Ave. value (ng/mL)	1.44	21.76	1.27	23.38	1.53	23.32
CV(%)	4.9	7.3	10.8	10.9	6.2	3.6
Relative bias(%)	-4.0	-9.3	-15.5	-2.6	1.9	-2.8

7.2 Instrument Suitability

The kit is applicable to but not limited to the following instruments. The CV is not more than 20% and the relative bias is within ±20%.

Table 8. Instrument suitability results - Microplate Reader

Microplate Readers	MD Spectra Max ABS		Thermo Multiskan FC	
QCs	Sample (Low) (n=4)	Sample (High) (n=4)	Sample (Low) (n=4)	Sample (High) (n=4)
Theoretical Conc. (ng/mL)	1.5	24	1.5	24
Ave. value (ng/mL)	1.25	22.63	1.27	23.38
CV(%)	9.3	13.4	10.8	10.9
Relative bias(%)	-16.8	-5.7	-15.5	-2.6

■ CONCLUSION

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

■ REFERENCES

[1] USP <1225> Validation of Compendial Procedures

[2] USP <1103> Immunological Test Methods - Enzyme-Linked Immunosorbent Assay (ELISA)

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

[4] ChP <9012> Guidance for Method Validation of Quantitative Analysis of BiologicalSamples

[5] JP <G3-11-171> Enzyme-linked Immunosorbent Assay (ELISA)

Support & Contact

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