

Performance Verification Summary for Double-stranded RNA (dsRNA) ELISA Kit (Catalog No.: 36717ES)

Background

The Double-stranded RNA (dsRNA) ELISA Kit is independently developed by Yeasen Biotechnology (Shanghai) Co., Ltd., designed to detect residual levels of dsRNA, a byproduct generated during the in vitro transcription (IVT) process of mRNA. This kit utilizes a sandwich enzyme-linked immunosorbent assay (ELISA) principle combined with a biotin-streptavidin amplification system, enabling highly sensitive detection of residual dsRNA in samples. The data presented in this summary report originate from Yeasen Biotechnology (Shanghai) Co., Ltd. Validation parameters include specificity, precision, accuracy, sensitivity, and robustness.

This summary is provided for user reference only. Users are required to perform method validation for residual dsRNA detection according to their own experimental conditions, sample types, and regulatory or pharmacopoeial requirements.

Materials and Methods

1. Double-stranded RNA (dsRNA) ELISA Kit: Cat# 36717ES (YEASEN)
2. Raw Data: Electronically recorded in the sub-file "Product Experimental Report" under the master file "Double-stranded RNA (dsRNA) ELISA Kit".
3. Method: All materials and procedures were developed and established by Yeasen Biotechnology (Shanghai) Co., Ltd. The kit has undergone extensive testing and evaluation during development and meets the relevant requirements of ICH Q2(R1) and the Chinese Pharmacopoeia (2020 Edition), Part IV, "Guideline 9101: Analytical Method Validation".

Validation Parameters and Results

1. Detection Range

The kit includes four types of dsRNA standards. Users may select the standard type matching their process to generate a standard curve; it is not necessary to prepare and test all four. This report presents standard curves and corresponding performance verification data for all four dsRNA standard types.

STD1: Unmodified dsRNA standard

Linear range: 0.0156–1 pg/μL, $R^2 > 0.99$, CV < 10% across all tested concentrations.

Standard Concentration (pg/μL)	Mean Measured Value (pg/μL)	Recovery (%)	CV (%)
1	1.0001	100.0%	3.1%
0.5	0.4994	99.9%	2.4%
0.25	0.2516	100.7%	3.3%
0.125	0.1227	98.1%	0.5%
0.0625	0.0640	102.5%	3.2%
0.0312	0.0309	99.2%	1.3%

Standard Concentration (pg/μL)	Mean Measured Value (pg/μL)	Recovery (%)	CV (%)
0.0156	0.0157	100.4%	3.2%
0	/	/	/

Table 1. Standard Curve for STD1: Unmodified dsRNA

STD2: pUTP-modified dsRNA standard

Linear range: 0.0156–1 pg/μL, $R^2 > 0.99$, CV < 10%.

Standard Concentration (pg/μL)	Mean Measured Value (pg/μL)	Recovery (%)	CV (%)
1	1.0001	100.0%	0.7%
0.5	0.4994	99.9%	0.1%
0.25	0.2514	100.6%	0.9%
0.125	0.1232	98.6%	2.5%
0.0625	0.0635	101.6%	1.1%
0.0312	0.0312	99.9%	0.5%
0.0156	0.0155	99.6%	0.0%
0	/	/	/

Table 2. Standard Curve for STD2: pUTP-Modified dsRNA

STD3: N1-Me-pUTP-modified dsRNA standard

Linear range: 0.0312–2 pg/μL, $R^2 > 0.99$, CV < 10%.

Standard Concentration (pg/μL)	Mean Measured Value (pg/μL)	Recovery (%)	CV (%)
2	2.0031	100.2%	1.6%
1	0.9880	98.8%	2.4%
0.5	0.5188	103.8%	1.2%
0.25	0.2383	95.3%	2.2%
0.125	0.1242	99.4%	0.1%
0.0625	0.0629	100.7%	1.8%
0.0312	0.0361	115.7%	1.6%
0	/	/	/

Table 3. Standard Curve for STD3: N1-Me-pUTP-Modified dsRNA

STD4: 5-OMe-UTP-modified dsRNA standard

Linear range: 0.0312–2 pg/μL, $R^2 > 0.99$, CV < 10%.

Standard Concentration (pg/μL)	Mean Measured Value (pg/μL)	Recovery (%)	CV (%)
4	4.0096	100.2%	1.9%
2	1.9940	99.7%	0.7%
1	0.9977	99.8%	0.1%

Standard Concentration (pg/ μ L)	Mean Measured Value (pg/ μ L)	Recovery (%)	CV (%)
0.5	0.5108	102.2%	0.1%
0.25	0.2454	98.2%	5.6%
0.125	0.1207	96.5%	2.8%
0.0625	0.0624	99.9%	0.3%
0	/	/	/

Table 4. Standard Curve for STD4: 5-OMe-UTP-Modified dsRNA

2. Specificity

1) Specificity – Recognition of Different Nucleic Acid Types

The kit specifically recognizes dsRNA and shows no reactivity toward ssRNA, dsDNA, or ssDNA.

Test Category Antigen Concentration (pg/ μ L)	dsRNA	ssRNA	dsDNA	ssDNA
	OD Value			
16	3.284	0.086	0.081	0.085
8	2.128	0.089	0.084	0.083
4	1.252	0.082	0.081	0.085
0	0.084	0.077	0.086	0.081

Table 5. Antibody Recognition of Different Nucleic Acid Types

2) Specificity – Impact of dsRNA Length and Sequence

dsRNAs of different lengths and sequences were tested using each of the four standard types included in the kit.

Results: The assay values are largely unaffected by dsRNA sequence or length.

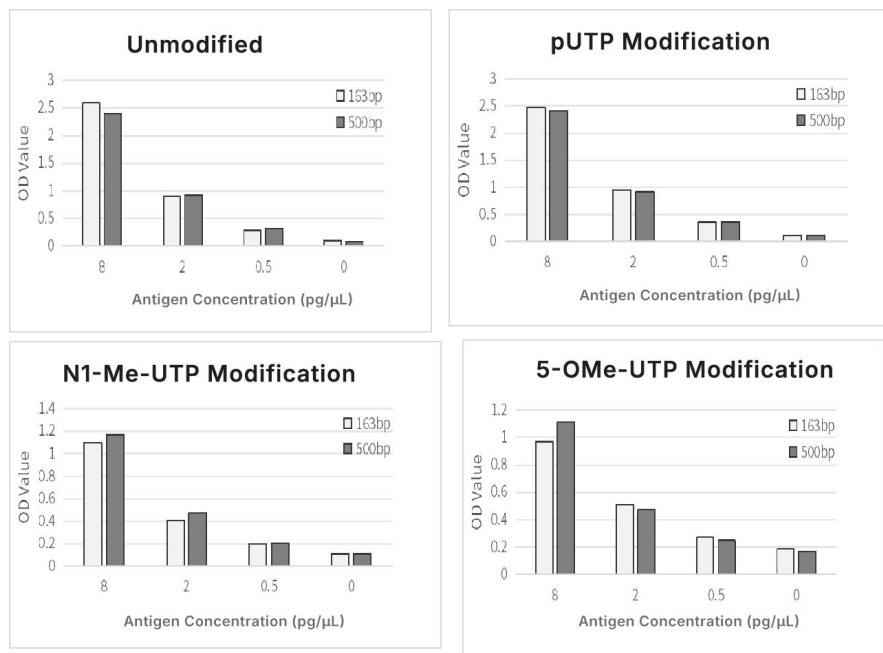


Figure 1. Testing Results for dsRNAs of Different Lengths and Sequences

3) Interference Testing

A customer-provided mRNA sample was spiked with various enzymes and buffers, including T7 RNA Polymerase, Murine RNase Inhibitor (RI), Inorganic Pyrophosphatase (IPPA), DNase I, Vaccinia Capping Enzyme (VCE), 2'-O-Methyltransferase (MT), 1× IVT buffer, and 1 mM sodium citrate. The N1-Me-pUTP-modified dsRNA standard was used to generate the standard curve and test samples. This evaluates whether the detection of dsRNA by the kit's antibody is affected by these common IVT components.

Results: The presence of the eight tested enzymes and buffers did not interfere with dsRNA detection, and recovery rates remained within the 80–120% range.

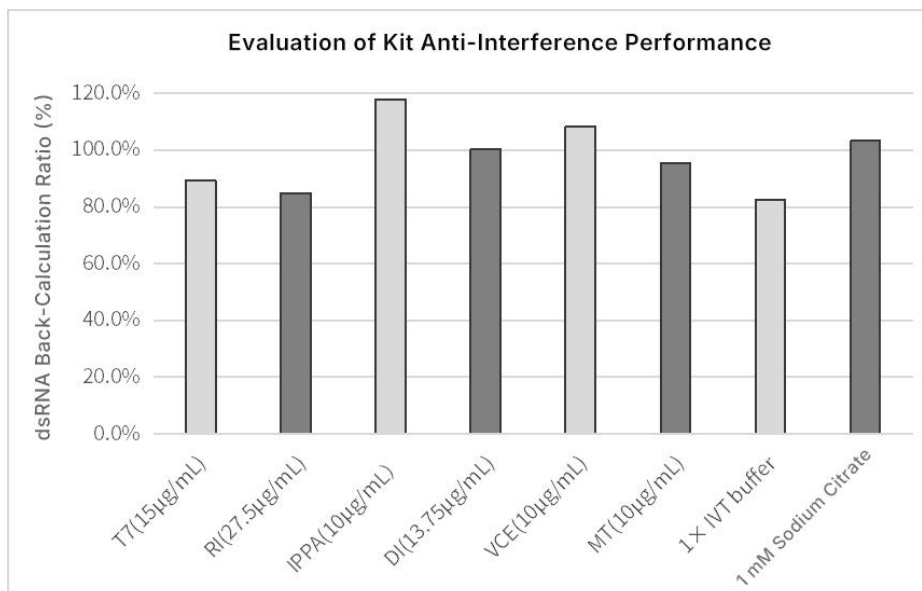


Figure 2. dsRNA Detection in mRNA Samples Spiked with Different Enzymes and Buffers

Sample Type	Measured dsRNA Concentration (pg/μL)	Recovery (%)
mRNA Sample	0.6057	100%
mRNA Sample + T7 RNA Polymerase (15 μg/mL)	0.5411	89.32%
mRNA Sample + Murine RNase Inhibitor (27.5 μg/mL)	0.5142	84.88%
mRNA Sample + IPPA (10 μg/mL)	0.7132	117.7%
mRNA Sample + DNase I (13.75 μg/mL)	0.6077	100.32%
mRNA Sample + VCE (10 μg/mL)	0.6563	108.3%
mRNA Sample + 2'-O-Methyltransferase (10 μg/mL)	0.5776	95.4%
mRNA Sample + 1× IVT Buffer	0.5003	82.6%
mRNA Sample + 1 mM Sodium Citrate	0.6251	103.2%

Table 6. dsRNA Measurement in mRNA Samples Spiked with Eight Different Enzymes and Buffers

3. Accuracy

To a known mRNA sample containing 0.36 pg/μL dsRNA, 0.5 pg/μL of unmodified dsRNA standard was spiked at three different volume ratios (1:1, 1:20, 1:250). The spike recovery met the acceptance criterion of 80–120%.

Spike Recovery = [(Measured Concentration after Spiking × Total Volume) – (Measured Sample Concentration × Sample Volume)] / (Theoretical Spiked Concentration × Spiked Volume) × 100%

Sample Type	Volume Ratio (Standard:Sample)	Measured dsRNA (pg/ μ L)	Spike Recovery (%)
Test Sample (TS)	/	0.36	/
TS + 0.5 pg/ μ L Unmodified dsRNA	1:1	0.769	81%
TS + 0.5 pg/ μ L Unmodified dsRNA	1:20	0.777	82%
TS + 0.5 pg/ μ L Unmodified dsRNA	1:250	0.803	82%

Table 7. Accuracy

4. Precision

1) Repeatability (Intra-assay Precision)

Three concentration levels (high, medium, low) of four dsRNA standards (unmodified, pUTP-modified, N1-Me-pUTP-modified, and 5-OMe-UTP-modified) were tested. Each concentration was measured in 8 replicates within a single assay. CV < 15%.

Unmodified dsRNA			
Theoretical Concentration (pg/ μ L)	Number of Replicates	Measured Concentration (pg/ μ L)	CV (%)
1	8	1.0002	3.11%
0.125	8	0.1230	0.46%
0.0156	8	0.0164	3.18%
pUTP-Modified dsRNA			
Theoretical Concentration (pg/ μ L)	Number of Replicates	Measured Concentration (pg/ μ L)	CV (%)
1	8	1.0001	0.65%
0.125	8	0.1231	2.46%
0.0156	8	0.0162	0.02%
N1-Me-pUTP-Modified dsRNA			
Theoretical Concentration (pg/ μ L)	Number of Replicates	Measured Concentration (pg/ μ L)	CV (%)
2	8	2.0022	1.58%
0.25	8	0.2444	2.17%
0.0312	8	0.0328	1.64%
5-OMe-UTP-Modified dsRNA			
Theoretical Concentration (pg/ μ L)	Number of Replicates	Measured Concentration (pg/ μ L)	CV (%)
8	8	8.0803	0.27%
1	8	0.9650	0.12%
0.125	8	0.1322	2.76%

Table 8. Repeatability

2) Intermediate Precision (Inter-assay Precision)

Three independent experiments were conducted using different kit lots. For each experiment, three concentration levels (high, medium, low) of the four dsRNA standards were tested, with 8 replicates per concentration. A total of 24 data points were obtained per concentration level. CV < 15%.

Unmodified dsRNA			
Theoretical Concentration (pg/μL)	Number of Assays	Measured Concentration (pg/μL)	CV (%)
1	3	1.0007	2.38%
0.125	3	0.1186	3.20%
0.0156	3	0.0173	1.27%
pUTP-Modified dsRNA			
Theoretical Concentration (pg/μL)	Number of Assays	Measured Concentration (pg/μL)	CV (%)
1	3	0.9987	0.09%
0.125	3	0.1269	1.06%
0.0156	3	0.0151	0.52%
N1-Me-pUTP-Modified dsRNA			
Theoretical Concentration (pg/μL)	Number of Assays	Measured Concentration (pg/μL)	CV (%)
2	3	2.0012	2.37%
0.25	3	0.2415	0.14%
0.0312	3	0.0330	1.81%
5-OMe-UTP-Modified dsRNA			
Theoretical Concentration (pg/μL)	Number of Assays	Measured Concentration (pg/μL)	CV (%)
8	3	7.9735	1.89%
1	3	1.0225	0.13%
0.125	3	0.1227	5.63%

Table 9. Intermediate Precision

5. Sensitivity

1) Limit of Detection (LOD)

The LOD is defined as the concentration corresponding to the mean of 24 blank (zero standard) OD values plus two times the standard deviation (Mean + 2SD), interpolated from the standard curve.

dsRNA Standard Type	Blank Mean (OD)	Blank SD (OD)	Mean + 2SD (OD)	LOD (pg/μL)
Unmodified	0.016	0.00048	0.01696	≤0.001
pUTP-Modified	0.016	0.00072	0.01744	≤0.001
N1-Me-pUTP-Modified	0.034	0.00119	0.03638	≤0.001

dsRNA Standard Type	Blank Mean (OD)	Blank SD (OD)	Mean + 2SD (OD)	LOD (pg/ μ L)
5-OMe-UTP-Modified	0.115	0.00290	0.1208	≤ 0.01

Table 10. Limit of Detection

2) Limit of Quantitation (LOQ)

The LOQ is defined as the lowest concentration on the standard curve diluted further such that the CV < 20%, which is taken as the LOQ.

dsRNA Standard Type	Mean Measured Concentration (pg/ μ L) (LOQ)	Number of Replicates	CV (%)	Recovery (%)
Unmodified	0.0156	24	<10%	80–120%
pUTP-Modified	0.0312	24	<10%	80–120%
N1-Me-pUTP-Modified	0.0156	24	<10%	80–120%
5-OMe-UTP-Modified	0.125	24	<10%	80–120%

Table 11. Limit of Quantitation

6. Durability

Performance after storage at 4°C for 7 days and at 37°C for 7 days was compared to baseline (T0). Differences were within $\pm 20\%$.

dsRNA Standard Type	Target Concentration (pg/ μ L)	Difference from T0 after 7 Days at 4°C (%)
Unmodified	1	10%
pUTP-Modified	1	5%
N1-Me-pUTP-Modified	2	7%
5-OMe-UTP-Modified	4	11%
dsRNA Standard Type	Target Concentration (pg/ μ L)	Difference from T0 after 7 Days at 37°C (%)
Unmodified	1	18%
pUTP-Modified	1	4%
N1-Me-pUTP-Modified	2	5%
5-OMe-UTP-Modified	4	8%

Table 12. Robustness

References

1. Chinese Pharmacopoeia Commission (ChPC). Chinese Pharmacopoeia, Part IV: Guideline for Validation of Quantitative Analytical Methods for Biological Samples [S]. Beijing: China Science and Technology Press, 2020: 542–543.
2. NMPA, 2007: General Principles for Technical Review of Analytical Method Validation in Biologics Quality Control.
3. ICH (2022). Analytical Method Validation Q2, Draft Version.