dsRNA Detection Kit (ELISA)

ARETERNA
A Synthgene Company

Cat. No.: DS0001

PRODUCT DESCRIPTION

The process of preparing mRNA using T7 RNA polymerase can generate double-stranded RNA (dsRNA) impurities, which may trigger downstream innate immune responses, and potentially compromise the efficacy of mRNA therapeutics. Therefore, dsRNA detection is a crucial aspect of mRNA quality control.

The dsRNA Detection Kit (ELISA) utilizes a quantitative sandwich immunoassay technique. This assay is designed with two dsRNA-specific antibodies, enabling sensitive and selective detection of dsRNA molecules (≥60 bp) regardless of their nucleotide composition and sequence. The microwells of ELISA plate are pre-coated with one anti-dsRNA antibody to capture dsRNA. When samples or dsRNA standards are pipetted into the microwells, the dsRNA binds to the capture antibody. Then, biotin-labelled anti-dsRNA antibody is added to the microwells followed by streptavidin labeled HRP and TMB. The resulting color development is directly proportional to the amount of dsRNA present in the sample.

To ensure accurate detection of dsRNA from mRNA with various base modifications, the kit includes four dsRNA standards – wild-type uridine, N1-mehtyl-pseudo-uridine, 5-methoxy-uridine and pseudo uridine. Users are advised to match the dsRNA standard with their mRNA sample type for accurate measurement.

PRODUCT FEATURES

- **High Flexibility** – 4 types of dsRNA standards included in the kit, for precise quantification of various modification types.
- **High Sensitivity** – detection limit as low as 0.001 pg/μL.
- **High Specificity** – specifically recognizes dsRNA, no affinity with other types of nucleic acid fragments.
- **Easy Operation** – pre-coated, no need for self-coating.
- **High Accuracy** – can detect dsRNA of 60bp and above.

PRODUCT PERFORMANCE

Standard Types	Linearity Range (Correlation Coefficient >0.99)	Quantification Limit (CV<10%, Recovery Rate 80-120%)	Detection Limit	Recovery Rate	CV
Unmodified	0.0156-0.5 pg/μL	0.0156 pg/μL	0.001 pg/μL	80-120%	<10%
N1-Me-pUTP Modification	0.0312-1 pg/μL	0.0312 pg/μL	0.001 pg/μL		
pUTP Modification	0.0156-0.5 pg/μL	0.0156 pg/μL	0.001 pg/μL		
5-OMe-UTP Modification	0.0625-1 pg/μL	0.0625 pg/μL	0.01 pg/μL		

Areterna-LLC
sales@areterna.com
15810 Gaither Drive, Suite 230
Gaithersburg, MD 20877

ARETERNA LLC ·15810 Gaither Drive, Suite 230, Gaithersburg, MD 20877

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EXPERIMENTAL DATA

1. Standard Curve Data

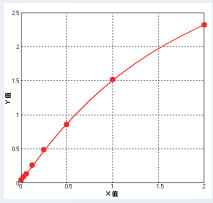
Standard curves were established for different types of dsRNA standards (dsRNA(N1-Me-pUTP), dsRNA(pUTP), and dsRNA (UTP)), and corresponding performance verifications were conducted. The results showed: $R^2 > 0.99$.

dsRNA(N1-Me-pUTP) Standard Curve

<>	dsRNA Standard Curve		Concentration (pg/μL)	Mean
A	2.3474	2.295	2	2.3212
B	1.5089	1.5262	1	1.51755
C	0.8708	0.8366	0.5	0.8537
D	0.4853	0.4813	0.25	0.4833
E	0.258	0.2564	0.125	0.2572
F	0.1332	0.1322	0.0625	0.1327
G	0.0871	0.0836	0.03125	0.08535
H	0.0387	0.0363	0	0.0375

Four-parameter logistic curve fitting
Equation: y = (A - D) / [1 + (x/C)^B] + D

A = 4.49500
B = -1.09578
C = 1.90869
D = 0.03718
r^2 = 0.99986

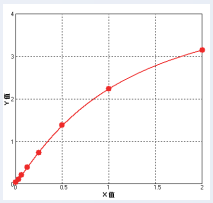


dsRNA(UTP) Standard Curve

<>	dsRNA Standard Curve		Concentration (pg/μL)	Mean
A	3.0421	3.2491	2	3.1456
B	2.2153	2.2497	1	2.2325
C	1.3661	1.4059	0.5	1.386
D	0.7431	0.7339	0.25	0.7385
E	0.3895	0.3937	0.125	0.3916
F	0.2165	0.2122	0.0625	0.21435
G	0.1073	0.1111	0.03125	0.1092
H	0.0387	0.0363	0	0.0375

Four-parameter logistic curve fitting
Equation: y = (A - D) / [1 + (x/C)^B] + D

A = 4.73792
B = -1.14794
C = 1.11815
D = 0.03843
r^2 = 0.99994

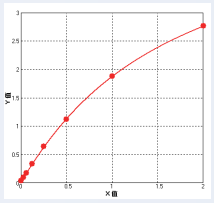


dsRNA(pUTP) Standard Curve

<>	dsRNA Standard Curve		Concentration (pg/μL)	Mean
A	2.7979	2.7268	2	2.76235
B	1.8466	1.9141	1	1.88035
C	1.1082	1.1396	0.5	1.1239
D	0.6477	0.6449	0.25	0.6463
E	0.3389	0.3349	0.125	0.3369
F	0.1728	0.1761	0.0625	0.17445
G	0.0948	0.0986	0.03125	0.0967
H	0.0387	0.0363	0	0.0375

Four-parameter logistic curve fitting
Equation: y = (A - D) / [1 + (x/C)^B] + D

A = 4.92200
B = -1.05855
C = 1.60121
D = 0.02931
r^2 = 0.99992



2. Sample Detection

Taking the dsRNA(N1-Me-pUTP) standard as an example, the residual dsRNA in the mRNA samples is consistently below 0.5%, meeting the quality control requirements and standards for mRNA bulk production.

Sample ID	Concentration (ng/μL)	Dilution	Well 1	Well 2	AVg. fluorescence -Y value	Calculated dsRNA residual amount pg/μL - X value	dsRNA Amount (ng/mg)	dsRNA residual rate %
1	1049.2	1000x	1.3003	1.3648	1.33255	446.271	425.34	0.0425
		2000x	0.8378	0.8392	0.8385			
2	1140.6	1000x	1.8088	1.7922	1.8005	567.36	497.42	0.0497
		2000x	1.0135	0.9875	1.0005			
3	698.5	1000x	1.736	1.7145	1.72525	532.0375	761.69	0.0762
		2000x	0.9457	0.8957	0.9207			
4	1184.7	1000x	0.7528	0.7339	0.74335	252.131	212.82	0.0213
		2000x	0.4357	0.3785	0.4071			
5	1098.8	1000x	0.5603	0.6803	0.6203	219.1895	199.48	0.0199
		2000x	0.3539	0.3519	0.3529			
6	1047.7	1000x	0.3568	0.3603	0.35855	124.155	118.50	0.0119
		2000x	0.2038	0.2066	0.2052			
7	1038.2	1000x	0.5714	0.5986	0.585	200.749	193.36	0.0193
		2000x	0.3161	0.3081	0.3121			

SUMMARY

The dsRNA Detection Kit (ELISA) utilizes two dsRNA-specific antibodies which allows sensitive and selective detection of dsRNA molecules. The kit comes with four dsRNA standards – wild-type uridine, N1-mehtyl-pseudo-uridine, 5-methoxy-uridine and pseudo uridine – which allows mRNA samples with different uridine modifications to be tested with better accuracy. Compared with dot-blot assays, the ELISA kit offers better data linearity and time savings.

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