Version 24-04/25

User's Manual

Double-stranded RNA (dsRNA) ELISA kit

REF **DEIA-BZ002P**

Σ **96T**

RUO

This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

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PRODUCT INFORMATION

Intended Use

This kit is used for quantitative detection of double stranded RNA (dsRNA) content in samples. The detected dsRNA is 40 bp or longer and is independent of its nucleic acid sequence.

General Description

mRNA has gained significant worldwide attention as a novel active ingredient in vaccines and gene therapies. The increasing demand for mRNA molecules has compelled mRNA manufacturers to quickly scale up production capacity while maintaining high mRNA quality. In vitro transcription by T7 polymerase is the standard procedure to synthesize mRNA. However, this procedure may introduce double-stranded RNA (dsRNA) contaminants from random priming of abortive transcripts, turn-around transcription, and antisense transcription. dsRNA immune activation results in the up-regulation of various pro-inflammatory cytokines, and cell death, which can lead to patient morbidity. Therefore, to improve the quality of mRNA translation, and minimize adverse effects, it is critical to carefully monitor in vitro-transcribed (IVT) mRNA products and confirm the removal of dsRNA after purification.

Principles of Testing

This kit is based on double antibody sandwich ELISA. Microplates are coated with dsRNA monoclonal antibodies to capture dsRNA antigens in the sample. After the detection antibody is added, the HRP conjugated antibody can be added to form a complex. After washing, the substrate solution was added to develop color. The chromogenic solution turns blue under the catalysis of HRP-conjugated antibody, and finally turns yellow under the action of acid. The depth of color is positively correlated with the amount of dsRNA in the sample.

Reagents And Materials Provided

1. Microtiter Plate;8×12 wells;2-8°C
2. Detection Antibody (100×);120 µL;2-8°C
3. HRP-Conjugated Antibody (100×);120 µL;2-8°C
4. Sample Diluent;30 mL;2-8°C
5. Substrate Solution;12 mL;2-8°C
6. Stop Solution;6 mL;2-8°C
7. Wash Buffer (20×);40 mL;2-8°C
8. Unmodified dsRNA Standard (5ng/µL);15µL;2-8°C
9. pUTP-modified dsRNA Standard (5ng/µL);15µL;2-8°C
10. N1-Me-pUTP-modified dsRNA Standard (5ng/µL);15µL;2-8°C
11. 5-OMe-UTP-modified dsRNA Standard (5ng/µL);15µL;2-8°C
12. STE buffer;50mL;2-8°C

13. Sealers;4 sheets;

Materials Required But Not Supplied

1. Microplate reader with 450±10nm filter(better if can detect at 450 and 650 nm wavelength).
2. Microplate shaker.
3. RNase-free tips and centrifuge tubes.

Storage

1. For unused kit: The whole kit could be stored at 2-8°C in shelf life. Strong light should be avoided for storage stability.

2. For used kit: Once the microplate is opened, please cover unused wells with plate sealer and return to the foil pouch containing the desiccant pack, zip-seal the foil pouch and return to 2-8°C as soon as possible after use. Other reagents should be returned to 2-8°C as soon as possible after use.

Reagent Preparation

1. Bring all kit components and samples to room temperature (18-25°C) before use. If the kit will not be used up in one time, please only take out strips and reagents for present experiment, and leave the remaining strips and reagents in required condition.
2. Wash buffer: dilute 40mL of 20×concentrated wash buffer with 760mL of deionized or distilled water to prepare 800mL of 1× wash buffer.
3. Standard: briefly spin or centrifuge the stock solution before use. The concentration of four standards provided is 5ng/μL. For standards, please dilute the stock solution to 2, 1, 0.5, 0.25, 0.125, 0.0625, 0.0312, 0pg/μL with STE buffer to draw the standard curve. We recommend standards can be diluted as following chart:

No.	Final Con. (pg/μL)	Dilution instruction	
		STE buffer	Standard
	100	49μL	1μL 5ng/μL standard
A	1	495μL	5μL 100pg/μL solution
B	0.5	250μL	250μL solution A
C	0.25	250μL	250μL solution B
D	0.125	250μL	250μL solution C
E	0.0625	250μL	250μL solution D
F	0.0312	250μL	250μL solution E
G	0.0156	250μL	250μL solution F
H	0	250μL	/

4. Biotinylated detection antibody and HRP-streptavidin working solution: briefly spin or centrifuge the stock solution before use. Dilute them to the working concentration with dilution buffer.
5. TMB substate: aspirate the needed dosage of the solution with sterilized tips and do not dump the residual solution into the vial again. TMB substate is sensitive to light, don't exposure TMB substrate to light for a long time.

Assay Procedure

1. Determine the number of strips required for the assay. Insert the strips in the frames for use. Remaining plate strips not used in this assay should be repacked in the bag with desiccant. Close the bag tightly for refrigerated storage.
2. Add 100μL each of dilutions of standard, blank and samples into the appropriate wells. Cover with the plate sealer. Incubate for 1hr at room temperature with shaking at 500rpm. The samples should be diluted with STE buffer to appropriate concentration for accurate assay.
3. Wash step: Aspirate the solution and wash with 250μL wash buffer to each well and let it stand for 30s. Discard wash buffer completely by snapping the plate onto absorbent paper. Totally wash 4 times.
4. Add 100μL of biotinylated detection antibody working solution into each well. Cover with the plate sealer. Incubate for 1hr at room temperature with shaking at 500rpm.
5. Repeat wash step.
6. Add 100μL of HRP-streptavidin working solution into each well. Cover with the plate sealer. Incubate for 30min at room temperature with shaking at 500rpm.
7. Repeat wash step again.
8. Add 100μL of TMB substrate solution into each well. Cover with the plate sealer. Incubate for 30 min at R.T. Protect from light. The liquid will turn blue by the addition of substrate solution.
9. Add 50μL of stop solution into each well. The liquid will turn yellow by the addition of stop solution. Then run the microplate reader and conduct measurement at 450nm immediately.

Calculation

1. Average the duplicate readings for each standard, control, and samples and subtract the average zero standard optical density. Construct a standard curve with absorbance on the vertical(Y) axis and dsRNA concentration on the horizontal(X)axis.
2. It is recommended to perform the calculation with computer-based curve-fitting software such as curve expert 1.3 or ELISA Calc in a 5 or 4 parameter non-linear fit model.

Typical Standard Curve

No.	Final Con. (pg/μL)	Dilution instruction	
		STE buffer	Standard
	100	49μL	1μL 5ng/μL standard
A	2	490μL	10μL 100pg/μL solution
B	1	250μL	250μL solution A
C	0.5	250μL	250μL solution B
D	0.25	250μL	250μL solution C
E	0.125	250μL	250μL solution D
F	0.0625	250μL	250μL solution E
G	0.0312	250μL	250μL solution F
H	0	250μL	/

No.	Final Con. (pg/μL)	Dilution instruction	
		STE buffer	Standard
	100	49μL	1μL 5ng/μL standard
A	4	480μL	20μL 100pg/μL solution
B	2	250μL	250μL solution A
C	1	250μL	250μL solution B
D	0.5	250μL	250μL solution C
E	0.25	250μL	250μL solution D
F	0.125	250μL	250μL solution E
G	0.0625	250μL	250μL solution F
H	0	250μL	/

Precision

CV of Intra-Assay ≤10%,CV of Inter-Assay ≤10%

Sensitivity

Limit of Detection

Unmodified dsRNA Standard (5ng/μL): ≤0.001pg/μL

pUTP-modified dsRNA Standard (5ng/μL): ≤0.001pg/μL

N1-Me-pUTP-modified dsRNA Standard: ≤0.001pg/μL

5- OMe-UTP-modified dsRNA Standard (5ng/μL): ≤0.01pg/μL

Limit of Quantitation

Unmodified dsRNA Standard (5ng/μL): 0.0156pg/μL

pUTP-modified dsRNA Standard (5ng/μL): 0.0156pg/μL

N1-Me-pUTP-modified dsRNA Standard: 0.0312pg/μL

5- OMe-UTP-modified dsRNA Standard (5ng/μL): 0.0625pg/μL

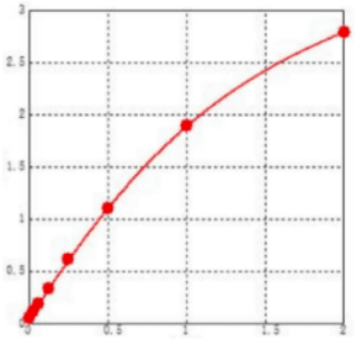
Specificity

This kit is for quantitative measurement of residual dsRNA.

Linearity

Liner detection range: 0.0312-1pg/μL

concentration (pg/μl)	N1-Me-pUTP modified dsRNA standard		
	OD450-OD650(1)	OD450-OD650(2)	AVERAGE
2	2.8412	2.7362	2.7887
1	1.8725	1.9135	1.8930
0.5	1.0863	1.1207	1.1035
0.25	0.623	0.6055	0.6143
0.125	0.3388	0.3292	0.3340
0.0625	0.1947	0.1885	0.1916
0.0312	0.1192	0.1247	0.1220
0	0.0567	0.0518	0.0543



Standard curve calculation

y = (A - D) / [(1 + (x/C)^B)]^n + D

A = 3.95937
B = -1.60805
C = 1.70005
D = 0.05238
n = 0.62358
r^2 = 0.99999

Recovery

80%~120%

Precautions

1. TMB reaction temperature and time is critical, please control them according to the instruction strictly.
2. In order to achieve good assay reproducibility and sensitivity, proper washing of the plates to remove excess un-reacted reagents is essential.
3. All the reagents should be mixed thoroughly prior to use and avoid bumbles during sample or reagents addition.
4. If crystals have formed in the concentrated wash buffer(20×), warm to 37°C and mix gently until the crystals are completely dissolved.
5. Avoid assay of samples containing Sodium Azide (NaN_3), as it could destroy the HRP activity resulting in under-estimation of the amount of dsRNA.
6. Avoid RNase contamination during assay.

7. The standard/sample, detection antibody and SA-HRP can also be conducted at R.T. without shaking, but this may cause detection sensitivity decrease by one-fold. For this case, we recommend N1-Me-pUTP dsRNA standards should be diluted from

4p g/μL. In addition, incubate HRP streptavidin working solution for 60min at room temperature. Do not use flask shaker, because flask shaker may result in inaccurate result.

8. If you need to detect UTP, pUTP, 5-OMe-UTP, please contact us. We will recommend a suitable kit for you.