Double-stranded RNA (dsRNA) ELISA Kit – 48 Tests

Ready-to-use kit for quantification of residual dsRNA.

Protocol

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Package content	**DSRNA-48T : Number of reactions : 48**
Shipping conditions	The kit is shipped in dry-ice.
Storage conditions	Upon receipt and for long-term use, store the kit at 4 - 8°C.
Stability	unopened product is valid 1 year* from the date of purchase. Once the reagent is opened, it is valid for half a year. *Upon receipt of the kit, please check whether all components are complete and immediately store them in corresponding condition.

For additional information and protocols (optimization, scaling, co-transfection…) tips, troubleshooting or other applications

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Any questions?

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Product description

Double-stranded RNA (dsRNA) emerges as a by-product during the in vitro transcription of mRNA. This by-product possesses immunogenic properties within the human body, capable of provoking an immune response, consequently diminishing mRNA levels. This renders dsRNA a troublesome process impurity necessitating thorough elimination and stringent control of its residual presence.

To address this concern, the Double-stranded RNA (dsRNA) ELISA detection kit employs the experimental principles of a double-antibody sandwich enzyme-linked immunoassay (ELISA) for the quantification of residual dsRNA. The Kit can not only be used in the detection of regular dsRNAs, but also in detection of peudo UTP, N1-Me-peudo UTP and 5-OMe-UTP modified dsRNAs.

The procedure involves adding both a standard and the test sample onto an enzyme plate (A) previously coated with anti-dsRNA antibodies. Subsequently, biotin-labeled dsRNA detection antibodies (F) are added in a diluted form. Finally, Streptavidin-HRP (SA-HRP) (G) is introduced to create a complex comprising antibodies, antigens, biotin, and SA-HRP.

Following plate washing, the addition of TMB chromogenic solution (K) initiates color development. Under the influence of HRP enzyme catalysis, TMB undergoes a transformation from colorless to blue, which is then halted through the application of the stop solution (L). Ultimately, this transformation results in a yellow hue, the intensity of which correlates positively with the quantity of dsRNA identified in the sample.

This kit serves a versatile purpose, enabling optimization of the biological product purification process, impurity control during intermediate stages, and release testing for final product assessment.

Components

Component	Name	Format
A	Anti-dsRNA coated microtiter strips	48 wells
B	STD1: unmodified, 5 ng/µL	7.5 µL
C	STD2: pUTP modified, 5 ng/µL	7.5 µL
D	STD3: N1-Me-pUTP modified, 5 ng/µL	7.5 µL
E	STD4: 5-OMe-UTP modified, 5 ng/µL	7.5 µL
F	Detection Antibody: Biotin-conjugated Ab (100x)	60 µL
G	Streptavidin-HRP (100x)	60 µL
H	Dilution Buffer 1	25 mL
I	Dilution Buffer 2	15 mL
J	Wash Buffer Concentrate (20×)	20 mL
K	TMB Substrate	6 mL
L	Stop Solution	3 mL
M	Plate Sealer	2 pieces

Protocol

1. Preparation before experiment

1) Experimental materials that are not provided in this kit but are required for the experiment:

a. RNase-free tips;

b. RNase-free centrifuge tube;

c. Dust-free paper;

d. RNase-free sample pretreatment plate;

e. Deionized water or double distilled water.

2) Instruments and equipment required but not provided:

a. Multifunctional microplate reader (including dual-wavelength detection mode, covering 450nm and 650nm; capable of using 450nm as the detection wavelength and 650nm as the reference wavelength);

b. Fully automatic plate washer, vortex mixer, constant temperature mixer, centrifuge;

c. Precision micropipettes and multi-channel micropipettes of different specifications;

d. Timer, 4℃ refrigerator;

e. Microplate shaker.

2. Assay method

1) Reagent preparation

Before experimenting, please equilibrate all reagents to room temperature (18~25°C), vortex and mix thoroughly, and then centrifuge momentarily to centrifuge the liquid on the tube cover or tube wall to the bottom of the tube.

* Dilution Buffer 1 (H) and Dilution Buffer 2 (I) do not require dilution.

a. 1×Wash Buffer preparation:

The concentrated solution is allowed to equilibrate to room temperature and be fully dissolved without crystallization. After mixing, dilute the concentrated washing solution (20×) 20 times with distilled water in a ratio of 1:20 according to the required amount, and finally get 1 × lotion. For example: take 50 mL of concentrated washing solution (20×) and add 950 mL of water to prepare 1000 mL of 1× washing solution.

b. Detection Antibody preparation:

Centrifuge at 1000 rpm for 30 seconds before use, and then use Dilution Buffer 2 to dilute the detection antibody to a working concentration of 1:100.

c. Streptavidin-HRP Preparation:

Centrifuge at 1000 rpm for 30 seconds before use, and then use Dilution Buffer 2 to dilute Streptavidin-HRP 1:100 to the working concentration.

2) Preparation of standard solution

Precautions before the experiment: 1. The sample addition operation should be as fast as possible. Too long time will affect the accuracy of the experiment; 2. All reagents should be thoroughly mixed before use. When adding samples, the sample should be added to the bottom of the well of the plate to avoid adding at the upper part of the well wall, be careful not to splash or generate bubbles when adding samples; 3. This kit provides 4 kinds of dsRNA standards. Users can choose modified standards that match their own processes for preparation, without the need for all preparation and testing.

Different dsRNA standards have different dilution concentration gradients, please follow the tables and dilute accordingly; 4. In each experiment, it's crucial to create a fresh standard curve that corresponds to the specific conditions of that experiment. It's important to emphasize that standard curves generated using different kits or at different times are not interchangeable or usable; 5. When conducting sample tests, dispense 100 µL of standard into each well. Ensure that you prepare an excess amount of standard to prevent any problems arising from insufficient quantities.

a. Dilution method for STD1 and STD2:

Please prepare 9 RNase-free 1.5 mL centrifuge tubes and label them according to the numbers provided in the Table 1 below. Take 1 µL of STD1 or STD2 and mix it with 49 µL of Dilution buffer 1 to create a standard solution with a concentration of 100 pg/µL. Add 495 µL of Dilution buffer 1 to tube A. Add 250 µL of Dilution buffer 1 to each of the remaining centrifuge tubes, labeled B-H.

Begin with tube A: Transfer 5 µL of the 100 pg/µL standard solution to tube A. Thoroughly mix to obtain a 1 pg/µL standard solution. Take 250 µL from tube A and transfer it to the next marked centrifuge tube. Repeat this process, performing a series of 2-fold gradient dilutions. The initial highest concentration is 1 pg/ µ L, and the lowest concentration is 0.0156 pg/µL.

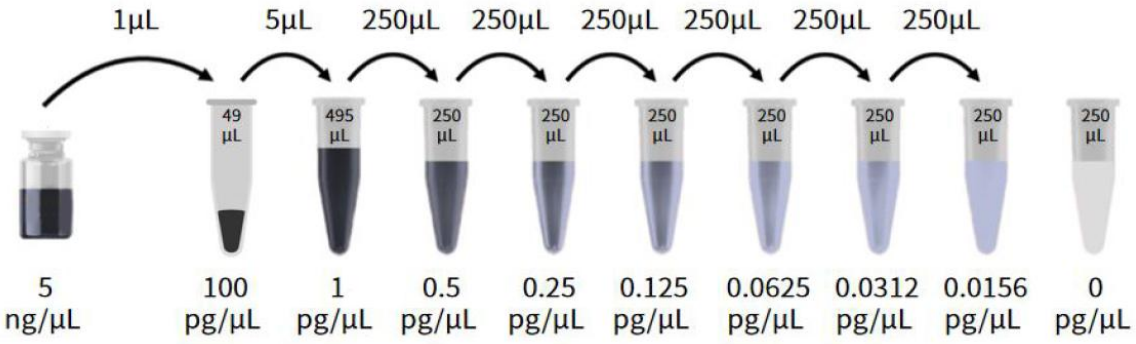

Figure 1. Flowchart for Standard STD1 and STD2 Preparation

Label	Vol. of Dilution Buffer 1 (µL)	Vol. of standards (µL)	Final conc. (pg/µL)
STD1/2	49	1 (STD1: unmodified, 5 ng/µL or STD2: pUTP modified, 5 ng/µL)	100
A	495	5 of STD1/2 (100 pg/µL)	1
B	250	250 of A	0.5
C	250	250 of B	0.25
D	250	250 of C	0.125
E	250	250 of D	0.0625
F	250	250 of E	0.0312
G	250	250 of F	0.0156
H	250	0	0

Table 1. Volume used in Diluting STD1 and STD2 Standards and the Final Concentration for Each Dilution Tier

b. Dilution method for STD3:

Please prepare 9 RNase-free 1.5 mL centrifuge tubes and label them according to the numbers provided in the table 2 below. Take 1 µ L of STD3 and mix it with 49 µ L of Dilution buffer 1 to create a standard solution with a concentration of 100 pg/µL. Add 490 µL of Dilution buffer 1 to tube A. Add 250 µL of Dilution buffer 1 to each of the remaining centrifuge tubes, labeled B-H.

Begin with tube A: Transfer 10 µL of the 100 pg/µL standard solution to tube A. Thoroughly mix to obtain a 2 pg/µL standard solution. Take 250 µL from tube A and transfer it to the next marked centrifuge tube. Repeat this process, performing a series of 2-fold gradient dilutions. The initial highest concentration is 2 pg/ µ L, and the lowest concentration is 0.0312 pg/µL.

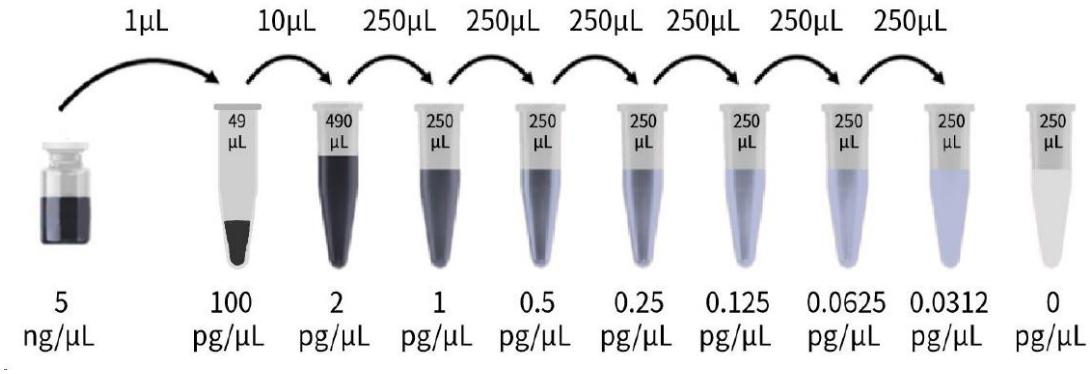

Figure 2. Flowchart for Standard STD3 Preparation

Label	Vol. of Dilution Buffer 1 (µL)	Vol. of standards (µL)	Final conc. (pg/µL)
STD3	49	1 of STD3: N1-Me-pUTP modified, 5 ng/µL)	100
A	490	10 of STD3 (100 pg/µL)	2
B	250	250 of A	1
C	250	250 of B	0.5
D	250	250 of C	0.25
E	250	250 of D	0.125
F	250	250 of E	0.0625
G	250	250 of F	0.0312
H	250	0	0

Table 2. Volume used in Diluting STD3 Standards and the Final Concentration for Each Dilution Tier

c. Dilution method for STD4:

Please prepare 9 RNase-free 1.5 mL centrifuge tubes and label them according to the numbers provided in the table 3 below. Take 1 µ L of STD4 and mix it with 49 µ L of Dilution buffer 1 to create a standard solution with a concentration of 100 pg/µL. Add 480 µL of Dilution buffer 1 to tube A. Add 250 µL of Dilution buffer 1 to each of the remaining centrifuge tubes, labeled B-H.

Begin with tube A: Transfer 20 µL of the 100 pg/µL standard solution to tube A. Thoroughly mix to obtain a 4 pg/µL standard solution. Take 250 µL from tube A and transfer it to the next marked centrifuge tube. Repeat this process, performing a series of 2-fold gradient dilutions. The initial highest concentration is 4 pg/ µ L, and the lowest concentration is 0.0625 pg/µL.

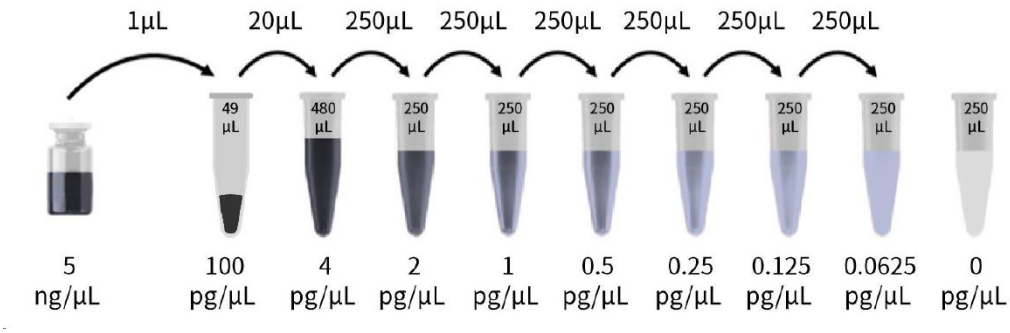

Figure 3. Flowchart for Standard STD4 Preparation

Label	Vol. of Dilution Buffer 1 (µL)	Vol. of standards (µL)	Final conc. (pg/µL)
STD4	49	1 of STD4: 5-OMe-UTP modified, 5 ng/µL)	100
A	480	20 of STD4 (100 pg/µL)	4
B	250	250 of A	2
C	250	250 of B	1
D	250	250 of C	0.5
E	250	250 of D	0.25
F	250	250 of E	0.125
G	250	250 of F	0.0625
H	250	0	0

Table 3. Volume used in Diluting STD4 Standards and the Final Concentration for Each Dilution Tier

3) Preparation of samples to be tested

Dilute the sample according to a certain dilution factor. The specific dilution factor of the sample needs to be spiked into the sample, and the appropriate dilution factor needs to be determined after evaluating the spike recovery rate and dilution linearity.

4) Experimental steps

Before commencing the assay, ensure that all reagent components and test samples have reached room temperature. It is highly recommended to perform all measurements on standards and test samples in duplicate.

a. Reagent Preparation: Prepare the necessary reagents, dilute standards, and test samples in advance.

b. Strip Preparation: Calculate the required number of strips for both the samples and standards. Remove the strips from the aluminum foil bag, return any unused strips to the bag, seal it, and store at a low temperature.

c. Sample Incubation: Add 100 µL of the standard and the test sample to each well. Ensure that sample addition is completed within 15 minutes. Seal the plate with a film, place it on a microplate shaker, and incubate at room temperature for 1 hour at 500rpm.

d. Microplate Cleaning: Discard the liquid in the wells, and wash the plate five times with 1×Wash Buffer (250 µL/well).

e. After each addition of wash buffer, let it sit for 30 seconds, then gently pat the microplate dry.

f. Biotin-Conjugated Antibody Incubation: Add 100 µ L of the pre-prepared detection antibodies at the working concentration to each well. Place the plate on a microplate shaker and incubate at room temperature for 1 hour..

g. Microplate Cleaning: Discard the liquid in the wells, and wash the plate five times with 1×Wash Buffer (250 µL/well) , and then pat the microplate dry.

h. Streptavidin-HRP Incubation: Add 100 µL of pre-prepared Streptavidin-HRP at the working concentration to each well. Place the plate on a microplate shaker and incubate at room temperature for 30 minutes at 500rpm.

i. Microplate Cleaning: Discard the liquid in the wells, and wash the plate five times with 1×Wash Buffer (250 µL/well) , and then pat the microplate dry.

j. Color Development: Allow the substrate solution (TMB) to reach room temperature for 10 minutes before use. Add 100 µL of TMB substrate solution to each well and incubate in the dark at room temperature for 30 minutes.

k. Termination: Add 50 µ L of stop solution to each well and gently shake the microplate to ensure even color development.

l. Absorbance Reading: Immediately read the absorbance values at 450nm (for detection) and 650nm (for reference) wavelengths.

5) Result analysis

- If the OD value of the sample to be tested exceeds the OD value at the highest point of the standard curve, the sample needs to be diluted and remeasured.
- Curve Development Procedure: Create a standard curve by plotting the standard concentrations on the x-axis and the corresponding calibrated standard absorbance values on the y-axis. Various drawing and statistical software tools can aid in the generation of standard curves and the determination of unknown sample concentrations. It is advisable to employ a four-parameter or five-parameter fitting method for this experiment.
- Ultimately, calculate the residual amount of dsRNA in the sample by referring to the standard curve and considering the sample's dilution factor.
- The provided standard curve is an illustrative example (for reference purposes only). Actual sample content should be determined using a standard curve specifically generated for the same experimental standard.

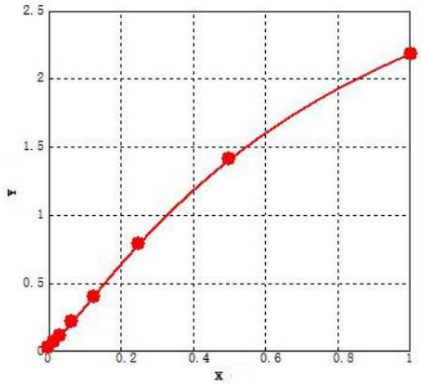

Figure 4. Standard curve

STD1 (pg/µL)	OD1	OD2	Av.
1	2.191	2.179	2.185
0.5	1.450	1.381	1.416
0.25	0.788	0.789	0.788
0.125	0.407	0.398	0.402
0.0625	0.225	0.221	0.223
0.0312	0.114	0.112	0.113
0.0156	0.069	0.070	0.070
0	0.028	0.029	0.028

Table 4. Standard OD value of unmodified STD1

6) Experiment flow chart

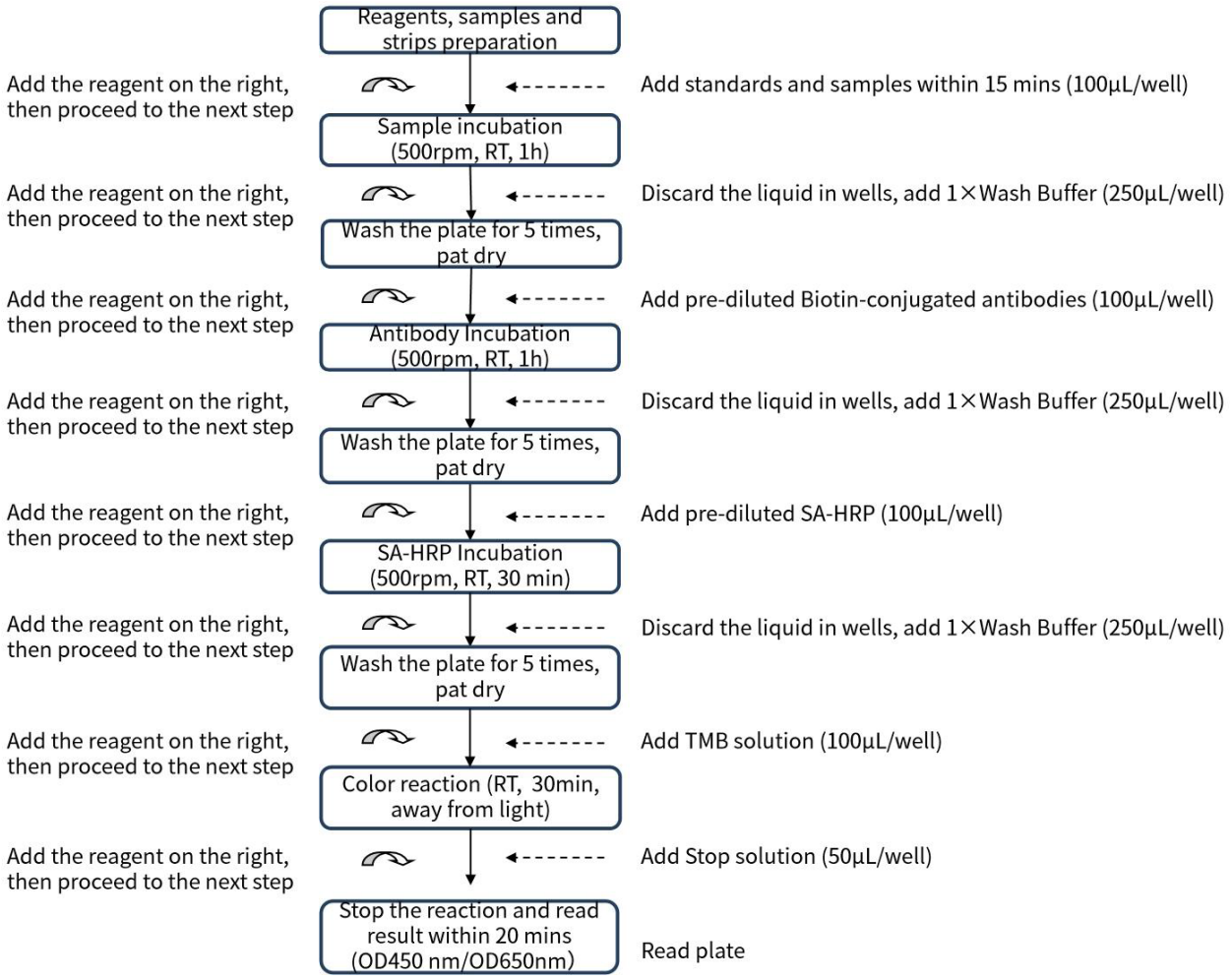

Figure 5. Experimental flow chart

Product performance

The performance of this kit has been fully evaluated. For details, please contact technical personnel to obtain the corresponding performance verification report.

Notes

1. Please read this instruction manual carefully before using this kit.
2. Please use this product within the validity period, and it is prohibited to mix related reagents from different batches.
3. All reagents need to be returned to room temperature before use.
4. For your safety and health, please wear a lab coat and disposable gloves.
5. This product is for scientific research purposes only.

Notes:

Notes:

Additional products for your mRNA production and transfection experiments :

- **IVT Kit** for mRNA synthesizing
- **Capping Kit** for the *In Vitro* enzymatic capping of RNA
- **Poly(A) tailing Kit** for RNA polyadenylation
- **RmesFect** for mRNA transfection

Purchaser Notification

This product is sold in accordance with our general conditions of sale that you can find on our website: https://ozbiosciences.com/content/3-terms-and-conditions.

Product Use Limitations

The **dsRNA ELISA Kit** and all its components are developed, designed, intended, and sold for research use only. They are not to be used for human diagnostic or included/used in any drug intended for human use. All care and attention should be exercised in the handling of the kit components by following appropriate research lab practices.
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