Double-stranded RNA (dsRNA) ELISA Kit

Product Description

dsRNA is a byproduct produced during the in vitro transcription of mRNA. It has strong immunogenicity and can easily induce an immune response in the body, thereby reducing the efficacy of mRNA biological products. Therefore, dsRNA is considered a process impurity that needs to be removed during the production process and its residual amount must be strictly controlled.

This kit adopts the principle of double antibody sandwich method and combines with Biotin-Streptavidin (B-SA) amplification detection system, which is specially used for quantitative detection of double-stranded RNA (dsRNA) (≥60 bp) in samples, regardless of the sequence of dsRNA. The detection process is as follows: first, the anti-dsRNA capture antibody is pre-coated in the microwells of the ELISA plate, and the sample to be tested is added and incubated to allow the dsRNA in the sample to specifically bind to the capture antibody. After washing to remove unbound substances, the biotin-labeled detection antibody is added for incubation to form a sandwich complex of "capture antibody-dsRNA-detection antibody". After washing again, horseradish peroxidase (HRP)-labeled streptavidin (SA) is added to achieve signal amplification through the high affinity binding of biotin-streptavidin. After thorough washing, the TMB substrate is added for color reaction. TMB appears blue under the catalysis of HRP and turns yellow after the reaction is terminated by acidic stop solution. The depth of the color is positively correlated with the content of dsRNA in the sample. Finally, the absorbance (OD value) of each well is measured at a wavelength of 450 nm using a Microplate reader, and the concentration of dsRNA in the sample is calculated based on the standard curve.

This kit has high sensitivity and simple operation process, and can be widely used in the optimization of biological product purification process, the control of dsRNA impurities in the intermediate process, and the release detection of the final product.

Composition and storage conditions

	48 Test	96 Test	Storage

Components \ Size			
ELISA Microplate	6 × 8 Well	12 × 8 Well	4 °C
Biotinylated detection antibody (100×)	60 μL	120 μL	4 °C
HRP-streptavidin (100×)	60 μL	120 μL	4 °C
Dilution buffer	15 mL	30 mL	4 °C
TMB substrate solution	6 mL	12 mL	4 °C
Stop solution	3 mL	6 mL	4 °C
Concentrated wash buffer (20×)	20 mL	40 mL	4 °C
dsRNA Standard (UTP, 5 ng/μL)	7.5 μL	15 μL	4 °C
dsRNA Standard (pUTP, 5 ng/μL)	7.5 μL	15 μL	4 °C
dsRNA Standard (N1-Me-pUTP, 5 ng/μL)	7.5 μL	15 μL	4 °C
dsRNA Standard (5-OMe-UTP, 5 ng/μL)	7.5 μL	15 μL	4 °C
STE buffer	25 mL	50 mL	4 °C
Plate sealer	2 pieces	4 pieces	4 °C

Shipping: Blue Ice **Shelf life:** 12 months

Experimental operation

1. Materials Not Supplied

(1) Microplate reader with 450±10nm filter (better if can detect at 450 and 650 nm wavelength), Microplate shaker (speed can reach 500 rpm), Centrifuge.

(2) RNase-free consumables (pipette tips, EP tubes, etc.) and RNase-free H_2O, absorbent paper, etc.

2. Preparation before detection

(1) Bring the kit components from the 4°C refrigerator and equilibrate at room temperature for 15 min.

***Note:** Please only take out strips and reagents for present experiment, and leave the remaining strips and reagents at 4°C. Be careful to prevent moisture from entering the ELISA plate due to loose sealing.

(2) Concentrated wash buffer (20×) is diluted with RNase-free H_2O at a volume ratio of 1:19 to make 1× wash buffer.

(3) Gently centrifuge the Biotinylated detection antibody tube, HRP-streptavidin tube, and Standard tube at 1000 rpm for 30 s before use to avoid residual reagents on the tube wall and tube cap.

(4) Dilute the Biotinylated detection antibody (100×) and HRP-streptavidin (100×) 100-fold to 1× with the Dilution buffer for later use.

(5) Preparation of standards: 1) Unmodified or pUTP-modified dsRNA standards is diluted with the STE buffer to 1, 0.5, 0.25, 0.125, 0.0625, 0.0312, 0.0156, 0 pg/μL; 2) N1-Me-pUTP-modified dsRNA standards is diluted with STE buffer to 2, 1, 0.5, 0.25, 0.125, 0.0625, 0.0312, 0 pg/μL; 3) 5-OMe-UTP-modified dsRNA standards is diluted with STE buffer to 4, 2, 1, 0.5, 0.25, 0.125, 0.0625, 0 pg/μL. The dilution method is as follows:

1) Preparation of unmodified or pUTP-modified dsRNA standards (detection linear range: 0.0156-0.5 pg/μL)

UTP/pUTP	STE buffer	Standard	Final Concentration (pg/μL)
	49 μL	1 μL 5 ng/μL standard	100
A	495 μL	5 μL 100 pg/μL stolution	1
B	250 μL	250 μL stolution A	0.5
C	250 μL	250 μL stolution B	0.25
D	250 μL	250 μL stolution C	0.125
E	250 μL	250 μL stolution D	0.0625
F	250 μL	250 μL stolution E	0.0312
G	250 μL	250 μL stolution F	0.0156
H	250 μL	/	0

2) Preparation of N1-Me-pUTP modified dsRNA standard (detection linear range: 0.0312-1 pg/μL)

N1-Me-pUTP	STE buffer	Standard	Final Concentration (pg/μL)
	49 μL	1 μL 5 ng/μL standard	100
A	490 μL	10 μL 100 pg/μL stolution	2
B	250 μL	250 μL stolution A	1
C	250 μL	250 μL stolution B	0.5

D	250 μL	250 μL stolution C	0.25
E	250 μL	250 μL stolution D	0.125
F	250 μL	250 μL stolution E	0.0625
G	250 μL	250 μL stolution F	0.0312
H	250 μL	/	0

3) Preparation of 5-OMe-UTP modified dsRNA standards (detection linear range: 0.0625-1 pg/μL)

5-OMe-UTP	STE buffer	Standard	Final Concentration (pg/μL)
	49 μL	1 μL 5 ng/μL standard	100
A	480 μL	20 μL 100 pg/μL stolution	4
B	250 μL	250 μL stolution A	2
C	250 μL	250 μL stolution B	1
D	250 μL	250 μL stolution C	0.5
E	250 μL	250 μL stolution D	0.25
F	250 μL	250 μL stolution E	0.125
G	250 μL	250 μL stolution F	0.0625
H	250 μL	/	0

***Note:** When diluting with a pipette, the sampling volume should be as accurate as possible. Errors in the sampling volume will lead to poor linearity of the standard curve and measurement errors of the sample.When performing gradient dilution of the standard, the sample in the high-concentration tube should be well mixed before taking the liquid and adding it to the low-concentration tube. Pay attention to avoiding cross-contamination.

3. Detection process

(1) Take out the required strips from the aluminum foil bag after equilibration at room temperature. Remaining plate strips not used in this assay should be repacked in the bag with desiccant. Close the bag tightly for refrigerated storage.

***Note:** Other components of the kit should be promptly returned to 4°C and used within the validity period.

(2) Set up standard wells and sample wells, add 100 μL of the standard solution of different concentrations

prepared above to each standard well, and add 100 μL of the sample to be tested to the sample well.

*Note: When the dsRNA content in the sample cannot be determined, it is recommended to use STE buffer to make multiple dilutions for testing to avoid the dsRNA concentration of the sample itself being too high to read the valid value. The linear range of UTP/pUTP detection is 0.0156-0.5 pg/μL, the linear range of N1-Me-pUTP detection is 0.0312-1 pg/μL, and the linear range of 5-OMe-UTP detection is 0.0156-0.5 pg/μL. It is recommended to perform 2 replicate wells for standards and samples and take the average value of the measurement.

(3) Seal the reaction wells with the Plate sealer and incubate for 60 min at room temperature with shaking at 500 rpm.

*Note: Be careful to avoid vigorous shaking that may cause the liquid in the microplate to splash out.

(4) Discard the liquid, pat dry on absorbent paper, and add 250 μL of 1× washing buffer to each well, let it stand for 30 s, then discard the washing solution and pat dry on absorbent paper. Repeat this washing process 4 times.

(5) Add 100 μL of the prepared 1×Biotinylated detection antibody to each well. Seal the reaction wells with the Plate sealer and incubate for 60 min at room temperature with shaking at 500 rpm.

*Note: Be careful to avoid vigorous shaking that may cause the liquid in the microplate to splash out.

(6) Discard the liquid, pat dry on absorbent paper, and add 250 μL of 1× washing buffer to each well, let it stand for 30 s, then discard the washing solution and pat dry on absorbent paper. Repeat this washing process 4 times.

(7) Add 100 μL of the prepared 1×HRP-streptavidin to each well. Seal the reaction wells with the Plate sealer and incubate for 30 min at room temperature with shaking at 500 rpm.

*Note: Be careful to avoid vigorous shaking that may cause the liquid in the microplate to splash out.

(8) Discard the liquid, pat dry on absorbent paper, and add 250 μL of 1× washing solution to each well, let it stand for 30 s, then discard the washing solution and pat dry on absorbent paper. Repeat this washing process 4 times.

(9) Add 100 μL of TMB substrate solution to each well, seal the reaction wells with the plate sealer, and incubate at room temperature for 30 min in the dark.

*Note: The TMB substrate solution should be added quickly to avoid oxidation, which may lead to abnormal measurement values. This incubation step is to place the reaction at room temperature away from light, without shaking; After adding the TMB substrate solution to the sample, the color of the liquid can be clearly seen to change from colorless to blue, which can be used to determine whether the experimental operation is normal.

(10) After the incubation is completed, add 50 μL of the Stop solution to each well, and then conduct the detection immediately. Set the wavelength of the microplate reader at 450 nm for the detection.

*Note: It is recommended that the microplate reader use dual wavelength 450 nm/650 nm for detection and use OD450-OD650 for calculation. nm detection can also be performed, and the blank signal value needs to be deducted when calculating the results.

4. Analysis of results

(1) Average the duplicate readings for each standard, control, and samples and subtract the average zero standard optical density. Draw the standard curve with the standard concentration as the horizontal axis (X) and the calibrated standard light absorption value as the vertical axis (Y).

*Note: The light absorption value in the dual-wavelength detection mode is 450 nm minus 650 nm.

(2) It is recommended to use professional curve making software, such as Curve Expert 1.3 or ELISA Calc (please use the Logistic five-parameter or four-parameter fitting curve calculation method to draw the standard curve, etc.) .

Notes

1. All reagents should be mixed thoroughly before use. Please add the samples to the bottom of the wells, and avoid adding them to the upper part of the well wall, be careful not to splash or generate bubbles. RNase contamination should be strictly avoided during the experimental operation.
2. The color development temperature and time are crucial to the experimental results and should be accurately controlled. During the washing process, the washing solution should soak the reaction plate for 30 s and then spin dry to fully wash out the non-specifically adsorbed components.
3. If crystals are found in the concentrated washing solution, incubate it in a 37°C water bath, and then mix and dilute to the working concentration after the crystals are completely dissolved.
4. Sodium azide (NaN_3) should be avoided from being introduced into the sample as it can destroy the activity of horseradish peroxidase, resulting in a lower detection value.
5. If the Microplate shaker is not available, room temperature static incubation can be adopted, but static incubation will cause the detection sensitivity to decrease by about double. When incubation at room temperature is adopted, it is recommended that unmodified or pUTP standards be diluted starting from 2 pg/μL, N1-Me-pUTP modified standards be diluted starting from 4 pg/μL, and 5-OMe-UTP modified standards be diluted starting from 8 pg/μL. The incubation duration of HRP-SA is adjusted from 30 minutes to 60 minutes. The remaining operations are consistent with the Microplate shaker operation.
6. This product is for scientific use only.

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