Instructions for Use of dsRNA ELISA Detection Kit

This kit is intended for scientific use and not for diagnostic use

Cat. No. HG- DS001

Product introduction

This kit, adopting the principle of double-antibody sandwich method and coupling with the streptavidin-biotin system, is used for quantitative detection of double-stranded RNA (dsRNA) content in samples. The length of dsRNA detected is 60 bp and above, and the dsRNA detected is independent of its nucleic acid sequence. Coat the microtiter plate wells with anti-dsRNA antibodies, add samples, incubate and wash, and then incubate with biotinylated detection antibody to form antibody-antigen-antibody complex, and streptavidin (SA) horseradish peroxidase (HRP) conjugate is added after washing again. After washing again, add streptavidin (SA) horseradish peroxidase (HRP) conjugate. After thorough washing, the TMB substrate is added for chromogenic reaction, and TMB is converted to blue under the catalysis of peroxidase and finally to yellow by the termination effect of acid. The shade of color is positively correlated with dsRNA content in the samples. The absorbance (OD value) is measured with a microplate reader at a wavelength of 450 nm, and the dsRNA concentration in the sample is calculated from the standard curve.

(1) Detection range:

The detection linear range of unmodified, pUTP-modified dsRNA is 0.0156-0.5 pg/μL

The detection linear range of N1-Me-pUTP-modified dsRNA is 0.0312-1 pg/μL

The detection linear range of 5-OMe-UTP-modified dsRNA is 0.0625-1 pg/μL

(2) Limit of detection (LoD):

The LoD of unmodified, pUTP-modified and N1-Me-pUTP-modified dsRNA is 0.001 pg/μL

The LoD of 5-OMe-UTP-modified dsRNA is 0.01 pg/μL

(3) Limit of quantitation (LoQ):

The LoQ of unmodified, pUTP-modified dsRNA is 0.0156 pg/μL

The LoQ of N1-Me-pUTP-modified dsRNA is 0.0312 pg/μL

The LoQ of 5-OMe-UTP-modified dsRNA is 0.0625 pg/μL

(4) Precision: CV% ≤ 10%

(5) Recovery: 80%-120%

Specification

96 T

Usage

It is suitable for quantitative detection of dsRNA content in samples.

Kit composition

Components	Specification	Preparation
Coated microtiter plate	8 × 12 plate strips	Ready-to-use
Biotinylated detection antibody (100×)	120 μL × 1 tube	Make a 100-fold dilution with diluent
streptavidin-HRP (100×)	120 μL × 1 tube	Make a 100-fold dilution with diluent
Diluent	30 mL × 1 bottle	Ready-to-use

Chromogenic solution	12 mL × 1 bottle	Ready-to-use
Stop solution	6 mL × 1 bottle	Ready-to-use
20× wash solution	40 mL × 1 bottle	Dilute with purified water at a volume ratio of 1:19 to obtain working solution for washing
dsRNA Standard (unmodified, 5 ng/μL)	15 μL × 1 tube	Dilute with STE buffer to obtain the required concentration
dsRNA Standard (pUTP-modified, 5 ng/μL)	15 μL × 1 tube	Dilute with STE buffer to obtain the required concentration
dsRNA Standard (N1-Me-pUTP-modified, 5 ng/μL)	15 μL × 1 tube	Dilute with STE buffer to obtain the required concentration
dsRNA Standard (5-OMe-UTP-modified, 5 ng/μL)	15 μL × 1 tube	Dilute with STE buffer to obtain the required concentration
STE buffer	50 mL × 1 bottle	Ready-to-use
Sealing film	3 films	Ready-to-use
IFU	1 copy	Ready-to-use

Notes: Store the kit at 2℃-8℃.

Storage conditions and shelf life

The unopened kit is valid for 12 months at 2℃-8℃.

Materials to be self-prepared

(1) Microplate reader

(2) Microtiter plate constant temperature oscillator

(3) Pipettes and disposable tips (0.5-10μL,10-100μL,30-300μL,100-1000μL)

(4) Deionized water

(5) Absorbent tissue

(6) EP tubes

Preparation before experiment

(1) Equilibrate the reagents used in this experiment to room temperature (18-25°C).

(2) Dilute 20× concentrated wash solution with purified water at a volume ratio of 1:19 to obtain washing working solution.

(3) Centrifuge the antibody tube, HRP-SA tube and standard tube at 1000 rpm for 30 s before use to avoid residual reagents on the tube wall and cap.

(4) 100× biotinylated detection antibody and 100× streptavidin-HRP are diluted 100-fold with diluent before use.

(5) Dilute unmodified, pUTP-modified dsRNA Standards to 1, 0.5, 0.25, 0.125, 0.0625, 0.0312, 0.0156, and 0 pg/μL with STE buffer.

Dilute N1-Me-pUTP-modified dsRNA Standards to 2, 1, 0.5, 0.25, 0.125, 0.0625, 0.0312, 0 pg/μL with STE buffer.

Dilute 5-OMe-UTP-modified dsRNA Standards to 4, 2, 1, 0.5, 0.25, 0.125, 0.0625, 0 pg/μL with STE buffer

The standard dilution method is recommended as follows:

a). Unmodified, pUTP-modified

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

b). N1-Me-pUTP-modified

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

c). 5-OMe-UTP-modified

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

Test steps

(1) Remove the required strips from the aluminum foil pouch after room temperature equilibration, seal the remaining strips with a sealing film and return to store at 2-8℃.

(2) Set the standard wells and sample wells, add 100 μL of standards at different concentrations to each standard well, and add 100 μL of samples to be tested to each sample well.

* When it is not possible to determine the dsRNA content in the sample to be tested, dilution factors shall be made with the STE buffer for the detection to avoid excessive content and inability to read valid values.

(3) Seal the reaction wells with a sealing film and shake the plate (500 rpm) at room temperature for 60 min.

(4) Discard the liquid, pat dry on absorbent tissue, fill each well with wash solution (250 μL), stand for 30 s, shake off the wash solution, pat dry on absorbent tissue, and repeat washing the plate 4 times in this way.

(5) Add 100 μL of biotinylated detection antibody at working concentration to each standard well and sample well. Seal the reaction wells with a sealing film and shake the plate (500 rpm) at room temperature for 60 min.

(6) Discard the liquid, pat dry on absorbent tissue, fill each well with wash solution (250 μL), stand for 30 s, shake off the wash solution, pat dry on absorbent tissue, and repeat washing the plate 4 times in this way.

(7) Add 100 μL of streptavidin-HRP at a working concentration to each standard well and sample well. Seal the reaction wells with a sealing film and shake the plate (500 rpm) at room temperature for 30 min.

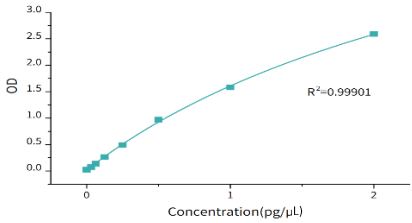
(8) Discard the liquid, pat dry on absorbent tissue, fill each well with wash solution (250 μL), stand for 30 s, shake off the wash solution, pat dry on absorbent tissue, and repeat washing the plate 4 times in this way.

(9) Add 100 μL of single-component substrate chromogenic solution to each well, seal the reaction wells with a sealing film, and allow them to stand at room temperature for 30 min in the dark.

(10) Add 50 μL of stop solution to each well and immediately perform the detection. Set the microplate reader wavelength at 450 nm (it is recommended to use dual-wavelength 450 nm/650 nm).

Result processing

Standard curve



Experimental Test Results

Antigen concentration	N1-Me-pUTP-modified standard		
(pg/μL)	OD (1)	OD (2)	Mean value
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

Precautions

(1) The temperature and time for chromogenic reaction are essential for the experimental results and shall be accurately grasped.

(2) During the washing process, the wash solution shall be soaked in the reaction plate for 30 s and then shaken dry to fully wash the non-specific adsorbed components.

(3) All reagents shall be fully shaken well before use, and the sample added shall be added to the medium and bottom of the wells of the microtiter plate during sample addition to avoid adding to the upper part of the well wall, and attention shall be paid not to splash and bubbles during sample addition.

(4) If crystals are found in the concentrated wash solution, they can be incubated in a 37°C water bath, mixed and diluted to the working concentration after the crystals are completely dissolved.

(5) The introduction of sodium azide (NaN_3) shall be avoided in samples, which may destroy horseradish peroxidase activity and lower the detection value.

(6) RNase contamination shall be strictly avoided during the experimental operation.

(7) A shaker shall not be used instead of an oscillator. If there is no oscillator, room temperature static incubation can be used, but static incubation will cause the detection sensitivity to decrease by about one time. It is recommended that the unmodified, pUTP-modified standard be diluted from 2 pg/μL, the N1-Me-pUTP-modified standard be diluted from 4 pg/μL, the 5-OMe-UTP-modified standard be diluted from 8 pg/μL, and the HRP-SA incubation time is adjusted from 30 min to 60 min.

Disclaimer

Under all circumstances, the liability of our company for this product is only limited to the value of the product itself.

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