Double-stranded RNA (dsRNA) ELISA kit

Cat.No.:HYJ521

Product Description

This kit is an Enzyme-Linked Immunosorbent Assay (ELISA) coupling with biotin-Streptavidin system, for quantitative measurement of dsRNA with length above 40 base pairs(bp), regardless of the sequence. The plate has been pre-coated with anti-dsRNA antibody. dsRNA present in the sample is added and binds to antibodies coated on the wells. And then biotinylated anti-dsRNA antibody is added and binds to dsRNA in the sample. After washing, HRP-Streptavidin is added and binds to the Biotinylated anti-dsRNA antibody. After incubation unbound HRP-Streptavidin is washed away. Then TMB substrate solution is added and catalyzed by HRP to produce a blue color product that changed into yellow after adding acidic stop solution. The density of yellow is proportional to the target amount of dsRNA captured in plate. The absorbance is measured at 450 nm.

Components

Components	48 T	96 T
ELISA Microplate	8×6	8×12
Biotinylated detection antibody (100x)	60 µL	120 µL
HRP-streptavidin (100x)	60 µL	120 µL
Dilution buffer	15 mL	30 mL
TMB substrate solution	6 mL	12 mL
Stop solution	3 mL	6 mL
Concentrated wash buffer (20x)	20 mL	40 mL
Standard (UTP, 1ng/µL)	7.5 µL	15 µL
Standard (pUTP,1ng/µL)	7.5 µL	15 µL
Standard (N1-Me-pUTP, 1ng/µL)	7.5 µL	15 µL
Standard (5-OMe-UTP, 1ng/µL)	7.5 µL	15 µL
STE buffer	25 mL	50 mL
Plate sealer	2 pieces	4 pieces

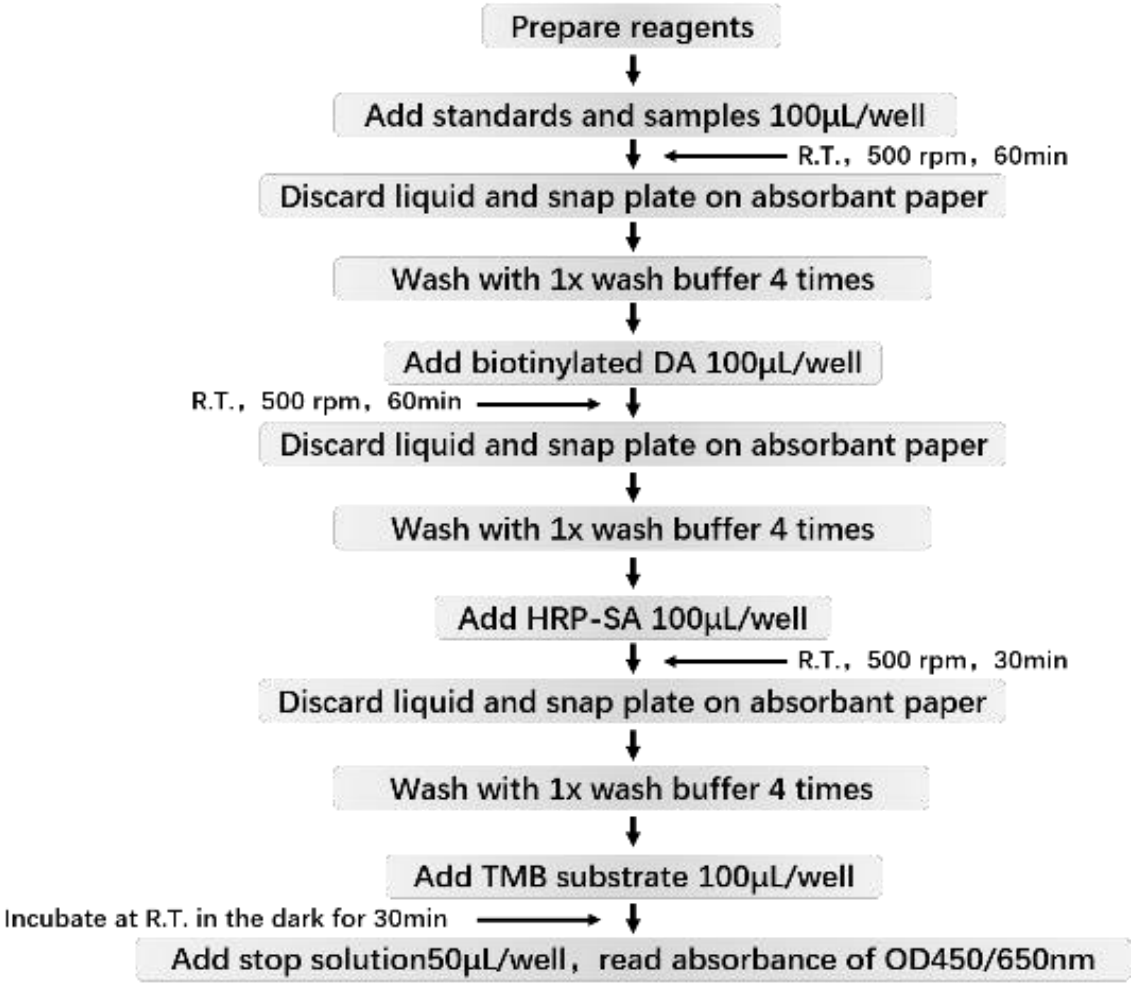
Storage

1. For unused kit: The whole kit could be stored at 2~8℃ 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℃ as soon as possible after use.
3. Other reagents should be returned to 2~8℃ as soon as possible after use.

Additional Materials Required

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.

Operation Flowchart



Instructions

1. Preparation before the experiment

1) Bring all kit components and samples to room temperature (18-25℃) 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 1ng/μL.

- For UTP dsRNA standards, please dilute the stock solution to 1, 0.5, 0.25, 0.125, 0.0625, 0.0312, 0.0156, 0pg/μL with STE buffer to draw the standard curve.
- For pUTP dsRNA standards, please dilute the stock solution to 0.5, 0.25, 0.125, 0.0625, 0.0312, 0.0156, 0.0078, 0pg/μL with STE buffer to draw the standard curve.
- For N1-Me-pUTP dsRNA standards, please dilute the stock solution to 1.5, 0.75, 0.375, 0.187, 0.093, 0.047, 0.023, 0pg/μL with STE buffer to draw the standard curve.
- For 5-OMe-UTP dsRNA standard, 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:

For UTP dsRNA standards

No.	Final Con. (pg/μL)	Dilution instruction	
		STE buffer	Standard
	10	99μL	1μL 1ng/μL standard
A	1	450μL	50μL 10pg/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	/

For pUTP dsRNA standards

No.	Final Con. (pg/μL)	Dilution instruction	
		STE buffer	Standard
	10	99μL	1μL 1ng/μL standard
A	0.5	475μL	25μL 10pg/μL solution
B	0.25	250μL	250μL solution A
C	0.125	250μL	250μL solution B
D	0.0625	250μL	250μL solution C
E	0.0312	250μL	250μL solution D
F	0.0156	250μL	250μL solution E
G	0.0078	250μL	250μL solution F
H	0	250μL	/

For N1-Me-pUTP dsRNA standards

No.	Final Con. (pg/μL)	Dilution instruction	
		STE buffer	Standard
	10	99μL	1μL 1ng/μL standard
A	1.5	425μL	75μL10pg/μL solution
B	0.75	250μL	250μL solution A
C	0.375	250μL	250μL solution B
D	0.187	250μL	250μL solution C
E	0.093	250μL	250μL solution D
F	0.047	250μL	250μL solution E
G	0.023	250μL	250μL solution F
H	0	250μL	/

For 5-OMe-UTP dsRNA standard

No.	Final Con. (pg/μL)	Dilution instruction	
		STE buffer	Standard
A	2	499μL	1μL 1ng/μL standard
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	/

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.

2. Detection steps

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.

3. Calculation of Results

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.

Performance

1. Sensitivity: lower limit of detection

≤0.001pg/μL (for UTP-, pUTP-, N1-Me-pUTP-dsRNA);

≤0.01pg/μL (for 5-OMe-UTP-dsRNA).

2. lower limit of quantitation

0.0156 pg/μL (for UTP-dsRNA)

0.0078 pg/μL (for pUTP-dsRNA)

0.023 pg/μL (for N1-Me-pUTP-dsRNA)

0.0312pg/μL (for 5-OMe-UTP-dsRNA)

3. Precision

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

4. Recovery

80%~120%

5. Linearity

0.0156-0.25pg/μL (for UTP-dsRNA)

0.0078-0.25pg/μL(for pUTP-dsRNA)

0.023-0.375pg/μL (for N1-Me-pUTP-dsRNA)

0.0312-0.5pg/μL (for 5-OMe-UTP-dsRNA)

Notes

1. TMB reaction temperature and time are critical, please control them according to the instruction strictly. But if the OD450 is beyond the microplate reader detection range, please reduce TMB reaction time properly.

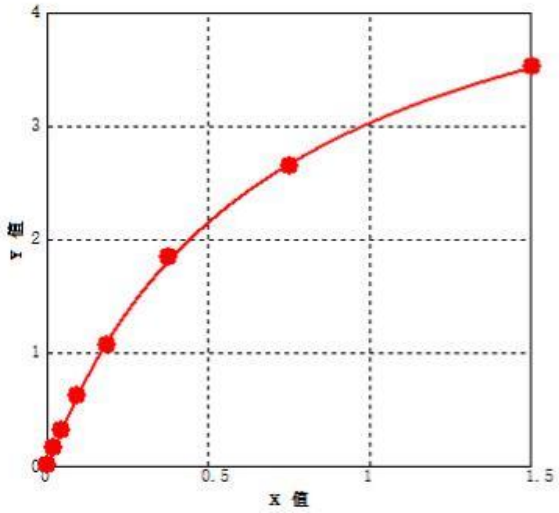
2. In order to achieve good assay reproducibility and sensitivity, proper washing of the plates to remove excess un-reacted reagents is essential.
3. All the reagents should be mixed thoroughly prior to use and avoid bumbles during sample or reagents addition.
4. If crystals have formed in the concentrated wash buffer(20x), warm to 37℃ 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 UTP dsRNA standards should be diluted from 2pg/μL, pUTP dsRNA standards should be diluted from 1pg/μL, N1-Me-pUTP dsRNA standards should be diluted from 3pg/μL and 5-OMe-UTP dsRNA standard should be diluted from 4pg/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.

Typical result

1. Standard curve data

Concentration (pg/μl)	N1-Me-pUTP modified dsRNA standard		
	OD450-OD650(1)	OD450-OD650(2)	AVERAGE
1.5	3.5318	3.5017	3.5168
0.75	2.6444	2.6594	2.6519
0.375	1.8339	1.8643	1.8491
0.187	1.0725	1.0771	1.0748
0.093	0.6176	0.6369	0.6273
0.046	0.3188	0.3203	0.3196
0.023	0.1766	0.1791	0.1779
0	0.0177	0.0171	0.0174

2. Standard curve calculation

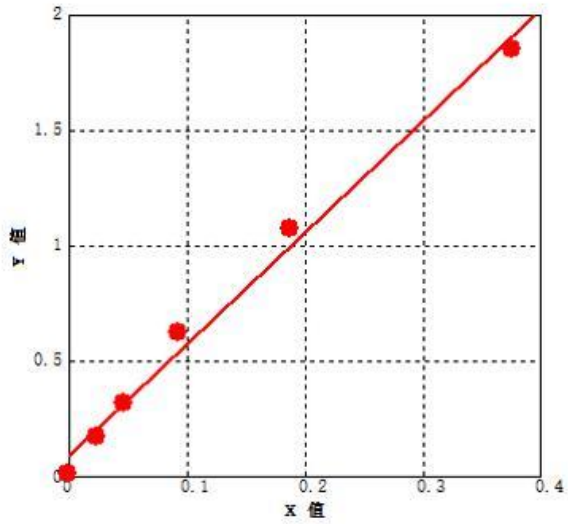


Standard curve calculation

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

A = 5.40641
B = -0.88200
C = 0.55048
D = 0.02079
n = 1.25330
r^2 = 0.99977

3. Liner detection range: 0.023-0.375pg/μL



Linear regression equation:

y = a + b*x

a = 0.09464
b = 4.83183
r^2 = 0.99026

4. Test example

	Sample concentration in assay	OD450-OD650	Calculated dsRNA concentration	Result judgement
eGFP	1ng/μl	1.7647	0.3587	valid
	0.4ng/μl	0.8998	0.1448	valid
Fluc	0.2ng/μl	0.4778	0.0701	valid
	4ng/μl	1.7403	0.3511	valid
	1.6ng/μl	0.8286	0.1312	valid
	0.8ng/μl	0.4534	0.0662	valid