EasyAna dsRNA (Modified) Quantitative Detection Kit (ELISA) 2.0

DD3509EN

Vazyme

Instruction for Use

Version 24.2

Contents

For Research Use Only. Not for use in diagnostic procedures.

01/Product Name

EasyAna dsRNA (Modified) Quantitative Detection Kit (ELISA) 2.0

02/Specification

96 tests

03/Intended Use

This kit employs double-antibody sandwich ELISA to detect the residual double-stranded RNA (dsRNA).

04/Test Principle

Messenger RNA (mRNA) is an intermediate molecule involved in protein synthesis that transmits genetic information encoded in DNA to cells. Thanks to mRNA technology advancement, including modifications to mRNA and improvements of delivery vectors, mRNA is being explored for various therapeutic purposes, including immuno-oncology targeted drugs, vaccines, proteins, and gene-editing drugs. The simplicity of the technique (i.e., in vitro synthesis of mRNA that is similar to endogenous mRNA and encodes the target protein, followed by delivery and expression in vitro or in vivo) makes it widely applicable.

The use of in vitro transcribed RNA for disease prevention or treatment requires a large number of functional mRNAs with low immunogenicity. Such mRNAs are synthesized mainly using bacteriophage T7 RNA polymerase (T7 RNAP), which can efficiently transcribe RNA from DNA templates containing specific promoters. However, previous studies have identified the presence of certain by-products that could trigger cellular immune responses during in vitro synthesis, including dsRNA, which has been proven to be a major trigger of immunity signaling pathways.

This kit employs double-antibody sandwich ELISA to detect the content of dsRNA in the in vitro transcription system or synthesized mRNA stock solution. Coat the microplate with capture antibodies to form solid-phase antibodies, add the dsRNA standard and the sample to be tested into the solid-phase antibody microplate, then add detection antibodies, and finally horseradish peroxidase (HRP)-labeled reagent to form "coating antibody-antigen-enzyme-labeled detection antibody" complexes. Add the TMB substrate solution after washing to develop color (the TMB substrate solution turns blue under the catalysis of HRP enzyme, and the color finally changes to yellow with an acid. The color intensity is positively correlated with the amount of dsRNA in the sample).

05/Key Components

Components	Cap Color	DD3509EN-01
1. Pre-coated microplate (coated with anti-dsRNA monoclonal antibody)	-	12 × 8, 96 wells
2. Sample diluent	-	2 × 30 ml
3. dsRNA (modified) standard (300 ng/ml)	Red	100 µl
4. Concentrated wash buffer (20×)	-	30 ml
5. Detection antibody (100×)	Blue	120 µl
6. Enzyme-labeled reagent diluent	-	12 ml
7. Enzyme-labeled reagent (100×)	Transparent	120 µl
8. TMB substrate solution	-	12 ml
9. Sealing film	-	4 pcs

Note: The components in this kit shall not be used interchangeably with those in other commercially available kits.

Reagents and consumables required but not provided:

>Deionized or distilled water

>Stop solution (component concentration): 0.5 M Sulfuric acid (H_2SO_4), 1.2 M Citric acid monohydrate ($C_6H_8O_7 \cdot H_2O$)

>Shaker

>Microplate washer (optional)

>Micropipettes and compatible sterile tips

>Thermostatic incubator or water bath

>Microplate reader

>Reagent reservoirs

>Absorbent pad

06/Storage

1. Store at 2 ~ 8℃ and protect from direct bright light. Ship on ice pack.
2. After a required number of pre-coated microplate strips are taken out, the remaining ones should be kept in the bag and stored at 2 ~ 8℃. The plate strips should be used within the shelf life.
3. The remainder of the other components in the kit should be put back at 2 ~ 8℃ quickly after use and should be used within the shelf life.
4. For product lot number and expiration date, see the label on the outer packaging.

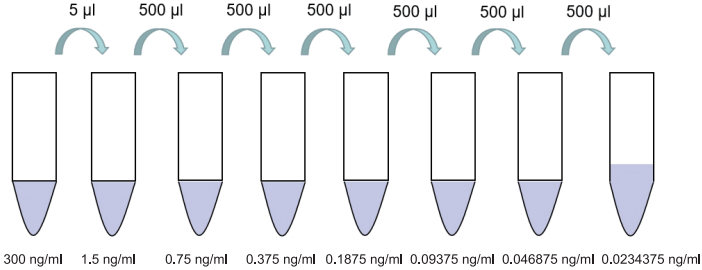
07/Test Method

07-1/Test Preparation

1. Take out the kit from the refrigerator and equilibrate it at room temperature (18 ~ 28℃) for at least 30 minutes.

2. Preparation of wash buffer (1×): Dilute the concentrated wash buffer (20×) with deionized water or distilled water by a factor of 20, and mix well for later use. For example, dilute 30 ml of concentrated wash buffer (20×) with 570 ml of deionized or distilled water.

3. Preparation of standard: Dilute the stock solution of the standard (300 ng/ml) by a factor of 200 to the first concentration point of the standard (1.5 ng/ml), and then continue serial dilution by a factor of 2 to 0.75、0.375、0.1875 、0.09375、0.046875 and 0.0234375 ng/ml respectively. To ensure the validity of the test results, please use a freshly prepared standard solution for each test.



Pipette	Into	Concentration of Prepared dsRNA Standard
5 µl of 300 ng/ml standard	995 µl of sample diluent	1.5 ng/ml
500 µl of 1.5 ng/ml standard	500 µl of sample diluent	0.75 ng/ml
500 µl of 0.75 ng/ml standard	500 µl of sample diluent	0.375 ng/ml
500 µl of 0.375 ng/ml standard	500 µl of sample diluent	0.1875 ng/ml
500 µl of 0.1875 ng/ml standard	500 µl of sample diluent	0.09375 ng/ml
500 µl of 0.09375 ng/ml standard	500 µl of sample diluent	0.046875 ng/ml
500 µl of 0.046875 ng/ml standard	500 µl of sample diluent	0.0234375 ng/ml
500 µl of sample diluent	Empty tube (control well)	0 ng/ml

4. Preparation of detection antibody (1×): Use the sample diluent to dilute the detection antibody (100×) to the detection antibody (1×). Determine the dilution volume (100 µl diluted antibody per well) according to the number of tests. For example, to test the whole plate, 10 ml of detection antibody (1×) is theoretically required, while 11 ml of detection antibody (1×) is actually prepared. In other cases, prepare 10% more of the theoretical volume required for the specific number of tests.

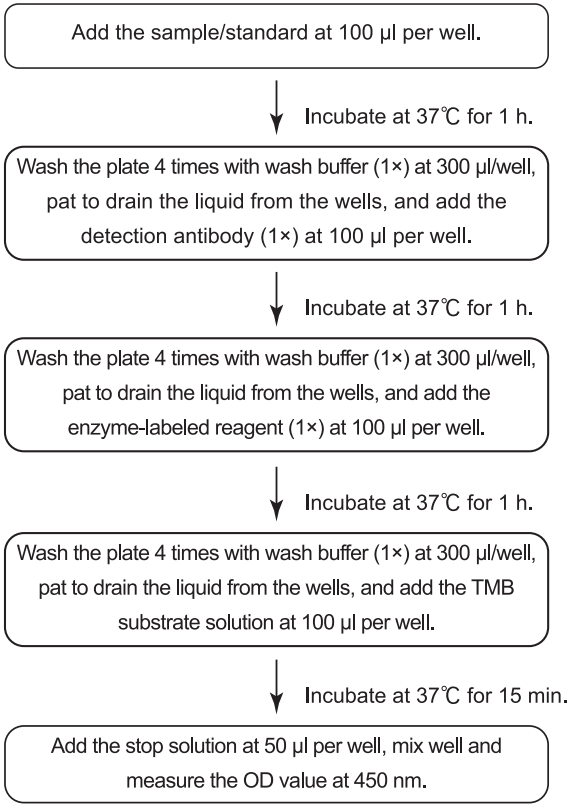
5. Preparation of enzyme-labeled reagent (1×): Use the enzyme-labeled reagent diluent to dilute the enzyme-labeled reagent (100×) to the enzyme-labeled reagent (1×). Determine the dilution volume (100 μl diluted reagent per well) according to the number of tests. For example, to test the whole plate, 10 ml of enzyme-labeled reagent (1×) is theoretically required, while 11 ml of enzyme-labeled reagent (1×) is actually prepared. In other cases, prepare 10% more of the theoretical volume required for the specific number of tests.

07-2/Test Operation

1. Sample loading: Add the sample/standard at 100 μl per well into the pre-coated microplate.
   ▲When the dsRNA content in the sample to be tested cannot be determined, use the sample diluent to perform serial dilutions by multiple dilution factors in case the content is too high to read a valid value.
2. Incubation: Seal the plate with a sealing film and incubate it in a 37℃ thermostatic incubator for 1 hour.
3. Plate washing: Upon the completion of incubation, carefully remove the sealing film, and discard the liquid in the wells. Add at least 300 μl of wash buffer (1×) into each well, and leave it to soak for 30 seconds. Wash the plate 4 times, and remove all of the residual liquid to the extent possible in the last washing.
4. Addition of detection antibody: Add the detection antibody (1×) at 100 μl per well.
5. Incubation: Seal the plate with a sealing film and incubate it in a 37℃ thermostatic incubator for 1 hour.
6. Plate washing: Upon the completion of incubation, carefully remove the sealing film, and discard the liquid in the wells. Add at least 300 μl of wash buffer (1×) into each well, and leave it to soak for 30 seconds. Wash the plate 4 times, and remove all of the residual liquid to the extent possible in the last washing.
7. Addition of enzyme-labeled reagent: Add the enzyme-labeled reagent (1×) at 100 μl per well.
8. Incubation: Seal the plate with a sealing film and incubate it in a 37℃ thermostatic incubator for 1 hour.
9. Plate washing: Upon the completion of incubation, carefully remove the sealing film, and discard the liquid in the wells. Add at least 300 μl of wash buffer (1×) into each well, and leave it to soak for 30 seconds. Wash the plate 4 times, and remove all of the residual liquid to the extent possible in the last washing.
10. Color development: Add the TMB substrate solution into the plate at 100 μl per well, seal the plate with a sealing film, and incubate it in a 37℃ thermostatic incubator for 15 minutes (keep away from light).

11. Termination/Reading: Add the stop solution at 50 μl per well, gently mix well and then use a microplate reader to measure the OD value per well at a single wavelength of 450 nm.

08/Brief Operating Procedure



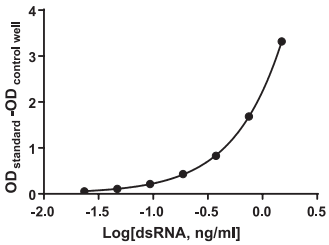
09/Quality Control

The correlation coefficient R^2 of the calibration curve should be ≥ 0.99; otherwise, the test is considered invalid.

10/Result Calculation

1. Subtract the OD value of the control well from the measured OD value of the standard and sample wells.
2. Plot the calibration curve by four-parameter fitting with the logarithm of the concentration of the standard as the x-coordinate and the OD value as the y-coordinate. If replicate wells are set, the calculation should be based on the mean value.
3. Substitute the OD value of the sample into the fitting equation of the calibration curve and calculate the sample concentration, i.e., the actual concentration of the sample. The limit of quantitation (LOQ) = 47 pg/ml. Values below 47 pg/ml should be reported as < 47 pg/ml. If the sample's OD value is above the upper limit of the calibration curve, a retest should be performed after appropriate dilution, and the concentration should be calculated by multiplying the dilution factor. The calibration curve below is for demonstration purposes only, and a new calibration curve should be generated for each test.

dsRNA Concentration (ng/ml)	$OD_{standard}$-$OD_{control\ well}$
1.5	3.3171
0.75	1.6846
0.375	0.8267
0.1875	0.4325
0.09375	0.2155
0.046875	0.1089
0.0234375	0.0565
0	0.0000
R^2	1



11/Performance Indicators

11-1/Sensitivity

LOD	LOQ
<10 pg/ml	47 pg/ml

11-2/Precision

	Within-Run Precision			Between-Run Precision		
Sample	Lot 1	Lot 2	Lot 3	Operator 1	Operator 2	Operator 3
n	3	3	3	3	3	3
Mean	1.2765	0.2009	0.0904	1.2099	0.1943	0.0823
SD	0.0732	0.0095	0.0051	0.0963	0.0158	0.0078
CV	6%	5%	6%	8%	8%	10%

11-3/Recovery

Sample (n = 3)	Measured Concentration (ng/ml)	Mean Measured Concentration (ng/ml)	Mean Recovery (%)	Recovery Range (%)
High	1.1120 1.1072 1.2780	1.1657	97	92-107
Medium	0.2067 0.1908 0.1770	0.1915	96	88-103
Low	0.0793 0.0712 0.0761	0.0756	94	89-99

12/Notes

1. Read the Instructions for Use carefully before operation and carry out the tests in strict accordance with it.
2. Avoid performing a test in harsh environments (e.g., environments containing dust and high-concentration corrosive substances such as chlorine-based disinfectant, sodium hypochlorite, acids, alkalis, or acetaldehyde). Disinfect the laboratory after the test. Do not perform tests in environments containing RNase III and UniversalBenzo Nuclease, which could degrade dsRNA.
3. Equilibrate the kit to room temperature before unpacking it, and shake the reagent well before use. Store and use each component in strict accordance with the Instructions for Use, and do not change or dilute the component arbitrarily. Carefully check the expiration date and packaging of the kit before use. If the kit expires or its package is damaged, do not use it for tests. Use the reagents within their shelf life, and seal and store the remainder according to the Instructions for Use.
4. The pre-coated microplate is removable. After taking off the required number of plate strips each time, keep the remainder in an aluminum foil pouch and store it at 2 ~ 8℃ for later use. Do not touch the bottom of the well when detaching the required strips from the plate to avoid fingerprints or scratches that may affect subsequent readings. After plate washing, immediately perform the next operation; otherwise, the plate may get dry and inactivated.
5. When loading the sample, avoid bubbles, and prevent the pipette tip from touching the bottom of the plate, which may cause scratches and affect the readings.
6. Do not re-use the sealing film. Do not use kit components with different lot numbers and micropipette tips interchangeably to avoid cross-contamination.

7. If crystals appear in the concentrated wash buffer, place the buffer at 37℃ until crystals are dissolved before use. Fill up each well with wash buffer during washing to ensure the residual reagent in each well is removed. Wash the plate thoroughly. Do not apply too much force when loading the solution to avoid solution contamination. Shake off the liquid from the wells after each plate washing (a plate washer is recommended), and pat it dry.
8. Read the result within 15 minutes after the reaction terminates.
9. Wear disposable gloves and protective gear in accordance with laboratory regulations during the operation. After the test, dispose of the liquid waste and disposable consumables in a harmless way in accordance with relevant local and national regulations.
10. Attention to adding sulfuric acid to the water and stiring with a glass rod when preparing the stop solution.

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