Cat.:BP-18-96

dsRNA Residual Quantitative Detection Kit 2.0

V01

Product Description

During the preparation of mRNA by in vitro transcription (IVT) using T7 RNA polymerase, by-products such as double-stranded RNA (dsRNA) are generated. dsRNA can not only trigger the body's innate immune response but may also lead to mRNA degradation. Therefore, the residual amount of dsRNA is a critical quality attribute for mRNA drugs. This kit employs a double-antibody sandwich enzyme-linked immunosorbent assay (ELISA) to detect the dsRNA content in in vitro transcription systems or synthesized mRNA stock solutions. Microplates are coated with capture antibody to form a solid-phase antibody. dsRNA standards and test samples are added to the solid-phase antibody microplate wells, followed by the addition of horseradish peroxidase (HRP)-labeled detection antibody, forming an antibody-antigen-antibody complex. After thorough washing, the TMB substrate is added. The substrate solution is converted to blue under the catalysis of HRP enzyme and finally to yellow under the action of acid. The intensity of the color is positively correlated with the amount of dsRNA in the sample.

Components

	Components	Volume	Storage
Box 1	dsRNA Standard (Unmodified UTP, 3 μg/mL)	30 μL×1 Tube	-20±5℃
	dsRNA Standard (pUTP-modified, 3 μg/mL)	30 μL×1 Tube	
	dsRNA Standard (N1-Me-pUTP-modified, 3 μg/mL)	30 μL×1 Tube	
	Detection Antibody	30 μL×1 Tube	
Box 2	Anti-dsRNA Antibody Precoated ELISA Plate	12×8 Wells, 96 Wells Total	2-8℃
	Concentrated Washing Buffer (20×)	20 mL×1 Bottle	
	Sample Diluent	30 mL×1 Bottle	
	Antibody Diluent	12 mL×1 Bottle	
	Chromogenic Solution	12 mL×1 Bottle	
	Stop Solution	6 mL×1 Bottle	
	Plate Sealer	4 Sheets	
	Manual	1 Copy	

Note: This kit shall not be mixed with other commercial kits.

Materials

Deionized water or distilled water, plate washer, micropipette, incubator, microplate reader and matching sterile pipette tips, reagent troughs, absorbent paper and other consumables.

Storage

1. Box 1 is stored at -20±5℃ and Box 2 at 2-8℃, protected from strong light.
2. After the precoated ELISA plate is unsealed and used, the remaining precoated ELISA plate shall be sealed, stored at 2-8℃ and used within the validity period.
3. Box 1 and Box 2 shall be promptly returned to the corresponding storage conditions after use.
4. Reagents in Box 1 shall be subjected to minimal repeated freeze-thaw cycles during use.

Protocol

1. Pre-Experiment Preparation

1.1 Take the kit out of the refrigerated environment and equilibrate at room temperature (18-28℃) for at least 30 minutes.

1.2 Prepare 1× Washing Buffer: Dilute the concentrated washing buffer (20×) 20-fold with deionized water or distilled water and mix well for use. For example, dilute 10 mL of concentrated washing buffer (20×) with 190 mL of deionized water or distilled water.

1.3 Preparation of Standards: Refer to Table 1 and Table 2 below for the preparation process. To ensure the validity of experimental results, prepare fresh standard solutions for each experiment. Due to differences in laboratory conditions, the standard curve response range may vary; the linear range can be adjusted according to experimental results to ensure more than 6 points for standard curve fitting. If the reactivity is too high, reduce the concentration of the first point of the

standard curve, and vice versa.

A: Unmodified and pUTP-modified dsRNA Standards: Dilute the standard stock solution (3 μg/mL) 1000-fold to obtain the first point of the standard (3 ng/mL), then perform 2-fold serial dilution to obtain 1.5, 0.75, 0.375, 0.187, 0.093, 0.047 ng/mL.

B: N1-Me-pUTP-modified dsRNA Standard: Dilute the standard stock solution (3 μg/mL) 500-fold to obtain the first point of the standard (6 ng/mL), then perform 2-fold serial dilution to obtain 3, 1.5, 0.75, 0.375, 0.187, 0.093 ng/mL.

Table 1 Preparation of Unmodified and pUTP-modified dsRNA Standards

Transfer	Add to	dsRNA Standard Concentration
1 μL of 3 μg/mL standard stock solution	999 μL of sample diluent	3 ng/mL
500 μL of 3 ng/mL standard	500 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	0.187 ng/mL
500 μL of 0.187 ng/mL standard	500 μL of sample diluent	0.093 ng/mL
500 μL of 0.093 ng/mL standard	500 μL of sample diluent	0.047 ng/mL
500 μL of sample diluent	Empty tube (control well)	0 ng/mL

Table 2 Preparation of N1-Me-pUTP-modified dsRNA Standards

Transfer	Add to	dsRNA Standard Concentration
2 μL of 3 μg/mL standard stock solution	998μL of sample diluent	6 ng/mL
500 μL of 6 ng/mL standard	500 μL of sample diluent	3 ng/mL
500 μL of 3 ng/mL standard	500 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	0.187 ng/mL
500 μL of 0.187 ng/mL standard	500 μL of sample diluent	0.093 ng/mL
500 μL of sample diluent	Empty tube (control well)	0 ng/mL

1.4 Prepare 1× Detection Antibody: Dilute the detection antibody with antibody diluent to prepare 1× detection antibody. For example, to prepare 1.7 mL, 4 μL of detection antibody is required. Determine the dilution volume according to the number of tests (100 μL per well). Ensure the detection antibody is fully mixed before sampling.

2. Detection Procedure

2.1 ELISA Plate Well Arrangement: Take out the corresponding number of plate strips from the aluminum foil bag according to the experimental volume, seal the remaining plate strips back into the aluminum foil bag and store at 2-8℃.

2.2 Sample Addition: Add 100 μL of sample/standard to each well of the ELISA plate, seal the plate with a plate sealer, and incubate in a 37℃ incubator for 1 hour. (If the approximate content of dsRNA in the test sample cannot be estimated, perform detection with multiple dilution factors using sample diluent to avoid invalid reading due to excessively high content).

2.3 Plate Washing: After incubation, carefully remove the plate sealer, discard the liquid in the wells, add at least 300 µL of 1× washing buffer to each well, let stand for soaking for 60 seconds, wash the plate 4 times continuously, and remove residual liquid as much as possible in the last wash.
2.4 Detection Antibody Addition: Add 1× detection antibody at a volume of 100 µL per well, seal the plate with a plate sealer, and incubate in a 37℃ incubator for 1 hour.
2.5 Plate Washing: After incubation, carefully remove the plate sealer, discard the liquid in the wells, add at least 300 µL of 1× washing buffer to each well, let stand for soaking for 60 seconds, wash the plate 4 times continuously, and remove residual liquid as much as possible in the last wash.
2.6 Color Development: Add 100 µL of TMB chromogenic solution to each well of the ELISA plate, seal the plate with a plate sealer, and incubate in a 37℃ incubator for 25 minutes in the dark.
2.7 Termination/Reading: Add 50 µL of stop solution to each well, mix gently, and detect the OD value of each well at a single wavelength of 450 nm with a microplate reader (dual wavelength 450nm/630nm is recommended). Complete the reading within 15 minutes.

Result Analysis

1. The OD values measured for standard and sample wells are corrected by subtracting the OD value of the control well for result calculation.
2. Plot the standard curve with the standard concentration as the abscissa and the OD value as the ordinate, fitted by the four-parameter method. If duplicate wells are set, the average value shall be used for calculation. For the test to be valid, the correlation coefficient R^2 of the standard curve shall be ≥0.99; otherwise, the experiment is invalid.
3. Substitute the OD value of the sample into the fitting equation of the standard curve to calculate the sample concentration, which is the actual concentration of the sample. If the OD value of the sample is higher than the upper limit of the standard curve, perform retesting after appropriate dilution, and multiply the calculated concentration by the dilution factor. A new standard curve shall be generated for each experiment. The standard curve plotted with theoretical concentration standards is shown in Figure 1 below.

dsRNA Concentration (ng/mL)	OD (Sample) - OD (Control Well)
3	2.787
1.5	1.732
0.75	0.966
0.375	0.503
0.1875	0.255
0.09375	0.143
0.046875	0.055
0	0

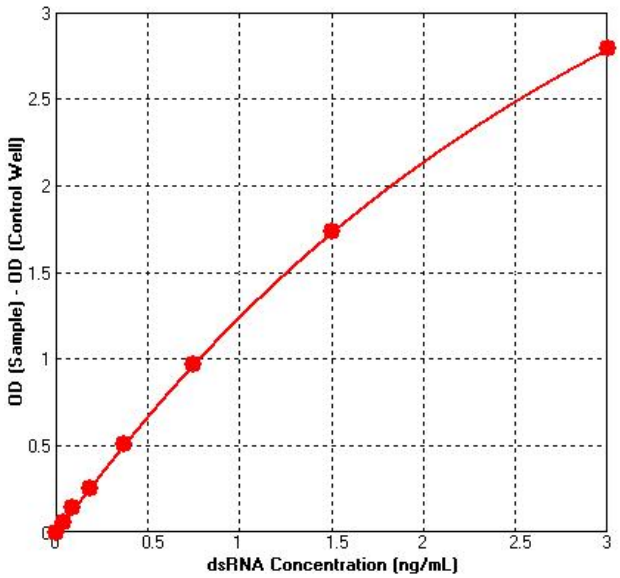

Figure 1 Standard Curve of dsRNA Standard (Unmodified UTP)

Product Performance

1. Precision: Intra-assay coefficient of variation < 10%; Inter-assay coefficient of variation < 15%.
2. Accuracy: 80%-120%.
3. Linear Range: The detectable linear range for unmodified and pUTP-modified dsRNA is 0.047-3 ng/mL; the detectable linear range for N1-Me-pUTP-modified dsRNA is 0.093-6 ng/mL.

Notes

1. Read the instruction manual carefully before operation and perform the experimental operation in strict accordance with the kit instruction manual.

2. Avoid conducting experiments in harsh environments (e.g., environments containing high-concentration corrosive gases such as 84 disinfectant, sodium hypochlorite, acid, alkali or acetaldehyde, and dust). Laboratory disinfection shall be carried out after the experiment is completed. RNase Ⅲ and benzonase can degrade dsRNA, so do not conduct experiments in environments containing RNase Ⅲ and benzonase.
3. Equilibrate the kit to room temperature before use, and shake the reagents thoroughly before use. After use, seal the remaining reagents in a timely manner and store them in accordance with the instruction manual.
4. The ELISA plate is detachable. After taking out the required number of plate strips each time, store the unused plate strips back in the aluminum foil bag at 2-8℃. Avoid scratching the bottom of the microplate reader or other operations that affect absorbance measurement.
5. The volumes of capture antibody, standard, detection antibody and SA-HRP are small; centrifuge the liquid on the tube wall and cap to the bottom of the tube with a centrifuge before use.
6. Plate sealers are for single use only. Do not mix reagent components of different batch numbers, and do not mix micropipette tips to avoid cross-contamination.
7. A plate washer is recommended for plate washing. Avoid cross-flow or air bubbles during the plate washing process to prevent affecting the accuracy of experimental results. After the last plate washing, pat dry the residual liquid in the wells.
8. Perform the reading within 15 minutes after reaction termination.
9. For your safety and health, wear disposable gloves and protective items specified by the laboratory during operation.
10. This product is for scientific research use only.