EasyAna T7 RNA Polymerase Quantitative Detection Kit (ELISA)

DD3504EN

Vazyme

Instruction for Use

Version 24.1

Contents

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

01/Product Name

EasyAna T7 RNA Polymerase Quantitative Detection Kit (ELISA)

02/Specification

96 tests

03/Intended Use

This kit employs double-antibody sandwich ELISA to detect the residual T7 RNA Polymerase.

04/Test Principle

The product T7 RNA Polymerase (T7 RNA polymerase) is a protein encoded by phage T7 DNA as expressed in recombinant *E. coli*, and is a kind of DNA-dependent 5'→3' RNA polymerase with high specificity for recognizing subsequence of T7 promoter. T7 RNA polymerase can contain single or double stranded DNA of the T7 promoter sequence as a template, and NTP as a substrate to synthesize RNA complementary to the single or double stranded DNA template downstream of the promoter.

This kit employs double-antibody sandwich ELISA to detect the content of T7 RNA Polymerase. The elisa plate is coated with monoclonal antibody against T7 RNA Polymerase to form the immobilized antibody. The T7 RNA Polymerase standard and the test sample are added to the immobilized antibody microplate. After washing, enzyme-labeled reagents labeled with horseradish peroxidase are added to form the "coated antibody - antigen - enzyme labeled detection antibody" complex.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 T7 RNA Polymerase in the sample) .

05/Key Components

	Component	Cap Color	DD3504EN-01
BOX 1	1. T7 RNA Polymerase standard (8,000 ng/ml)	Red	100μl
BOX 2	2. Pre-coated microplate(coated with anti-T7 RNA Polymerase monoclonal antibody)	-	12 ×8, 96 wells
	3. Sample diluent	-	2 × 30 ml
	4. Concentrated wash buffer (20 ×)	-	30 ml
	5. Enzyme-labeled reagent diluent	-	12 ml
	6. Enzyme-labeled reagent (100 ×)	Transparent	120 ul
	7. TMB substrate solution	-	12 ml
	8. Sealing film	-	3 pcs
	9. Instructions for Use	-	1 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：1M H_2SO_4, 1.2M $C_6H_8O_7 \cdot H_2O$

>Shaker

>Plate washer

>Micropipettes and compatible sterile tips

>Thermostatic incubator or water bath

>Microplate reader

>Sample loading slot

>Absorbent paper

06/Storage and Shelf Life

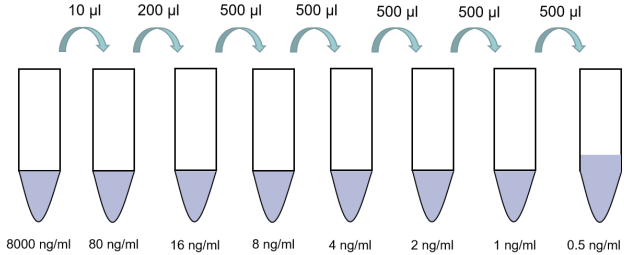
1. Store the BOX 1 at -30 ~ -15℃ and protect from direct bright light. The kit has a shelf life of 12 months.;
   Store the BOX 2 at 2 ~ 8℃ and protect from direct bright light. The kit has a shelf life of 12 months;
2. After a required number of pre-coated microplate strips are taken out, the remaining ones should be kept in the bag and stored at 2 ~ 8℃. The plate strips should be used within the shelf life.
3. BOX 1 After use, store it in a timely manner at -30 ~ -15 °C to avoid repeated freezing and thawing, and use it within its validity period.
4. BOX 2 After using other components, promptly return to the 2 ~ 8℃ condition and use within the validity period.
5. 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, remove the standard from the refrigerator, and equilibrate it at room temperature (18 ~ 28°C) 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 (8,000 ng/ml) 100 times to the concentration of 80 ng/ml, then dilute it 5 times as the first point of the standard (16 ng/ml), and then continue serial dilution by a factor of 2 to 8 ng/ml, 4 ng/ml, 2 ng/ml, 1 ng/ml, and 0.5 ng/ml,respectively. To ensure the validity of the text results, please use a freshly prepared standard solution for each test.

▲Please store the standards in different packages after first use



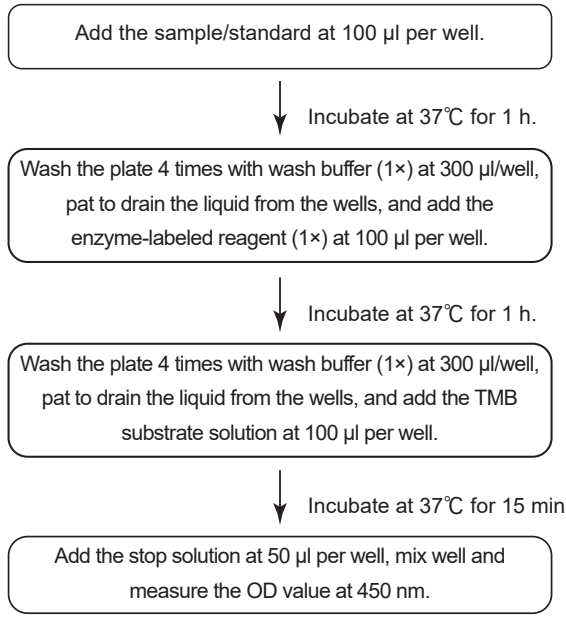
Pipette	Into	Concentration of Prepared T7 RNA Polymerase standard
10 µl of 8,000 ng/ml standard	990 µl sample diluent	80 ng/ml
200 µl of 80 ng/ml standard	800 µl sample diluent	16 ng/ml
500 µl of 16 ng/ml standard	500 µl sample diluent	8 ng/ml
500 µl of 8 ng/ml standard	500 µl sample diluent	4ng/ml
500 µl of 4 ng/ml standard	500 µl sample diluent	2 ng/ml
500 µl of 2 ng/ml standard	500 µl sample diluent	1 ng/ml
500 µl of 1 ng/ml standard	500 µl sample diluent	0.5 ng/ml
500 µl of sample diluent	Empty tube (control well)	0 ng/ml

4. 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.
   ▲Dilute the test sample to the mRNA concentration below 50 μg/ml by sample diluent.
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 enzyme-labeled reagent: Add the enzyme-labeled reagent (1×) at 100 μl per well.
5. Repeat steps 2 and 3.
6. 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).
7. 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



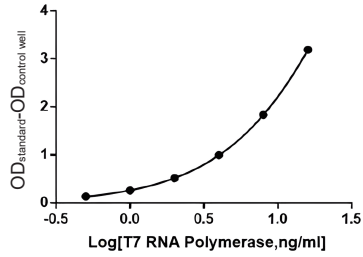
09/Quality Control

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. Take the logarithm of the concentration of the standard with a base of 10 as the x-axis and the OD value as the y-coordinate, and plot the calibration curve through four parameter method.If replicate wells are set, the calculation should be based on the mean value.
3. Calculate the sample concentration by subtracting the OD value of the control well from the OD value of the sample and substituting it into the fitting equation of the calibration curve, which is the actual concentration of the sample. The limit of quantitation (LOQ) = 0.5 ng/ml. Values below 0.5 ng/ml should be reported as < 0.5 ng/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.

T7 RNA Polymerase Concentration (ng/ml)	$OD_{standard}$-$OD_{control\ well}$
16	3.1923
8	1.8361
4	0.9950
2	0.5198
1	0.2628
0.5	0.1342
0	0.0000
R^2	1



11/Performance Indicators

11-1/Sensitivity

LOD	LOQ
100 pg/ml	0.5 ng/ml

11-2/Precision

	Within-Run Precision			Between-Run Precision		
Sample	Lot 1	Lot 2	Lot 3	Operator 1	Operator 2	Operator 3
n	8	8	8	8	8	8
Mean	2.3295	1.9380	1.8819	2.2142	2.1607	2.2902
SD	0.0827	0.0828	0.0394	0.1085	0.0721	0.1494
CV	4%	4%	2%	5%	3%	7%

11-3/Recovery rate

Sample (n=3)	Concentration (ng/ml)	Average measured concentration (ng/ml)	Average recovery rate %	Recovery rate range %
High	8.6875 9.0066 9.4987	9.0643	105	101-110
Medium	1.9855 2.0027 2.0334	2.0072	91	90-92
Low	0.7118 0.8122 0.7144	0.7461	102	97-111

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.
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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