Version 18-04/24

**User's Manual**

# T7 RNA Polymerase ELISA Kit

REF **DEIA-NS2308-1**

Σ **96T**

RUO

This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

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# PRODUCT INFORMATION

## Intended Use

It is suitable for the determination of T7 RNA Polymerase content in the samples.

## General Description

T7 RNA Polymerase (T7 RNA Polymerase) is a protein encoded by phage T7 DNA expressed by recombinant E.coli. It is a DNA-dependent 5'→3' RNA polymerase that highly specifically recognizes the T7 promoter sequence. T7 RNA polymerase can use single-stranded or double-stranded DNA containing the T7 promoter sequence as a template, and use NTP as a substrate to synthesize RNA complementary to the single-stranded DNA or double-stranded DNA template strand downstream of the promoter.

## Principles of Testing

This kit employs double-antibody sandwich ELISA to detect the content of T7 RNA Polymerase. Coat the microplate with anti-T7 RNA Polymerase antibodies, add the T7 RNA Polymerase Standard and the sample to be tested into the solid-phase antibody microplate, then add Anti-T7RNA Polvmerase (detection antibody), and finally add Streptavidin HRP 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 T7 RNA Polymerase in the sample).

## Reagents And Materials Provided

1. T7 RNA Polymerase Coated Plate, 8×12 wells.
2. Anti-T7 RNA Polymerase, 150μL.
3. Streptavidin HRP, 150μL.
4. T7 RNA Polymerase Standard, 30μL, 0.668mg/mL.
5. Sample Diluent Buffer, 60mL.
6. Antibody Diluent Buffer, 12mL.
7. Enzyme Conjugate Diluent Buffer, 12mL.
8. 20×PBST Wash Buffer, 50mL
9. TMB substrate solution, 12mL
10. Stop Solution, 7mL

## Materials Required But Not Supplied

1. Deionized water
2. Vortex shaker

3. Micropipettes and compatible sterile tips
4. Thermostatic incubator
5. Microplate reader
6. Filter paper

## Storage

Anti-T7 RNA Polymerase, Streptavidin HRP, T7 RNA Polymerase Standard: -18°C

Others: 2-8°C, Store away from light.

Shelf life: 12 months

## Reagent Preparation

**Note: Take out the kit from the refrigerator and the standard from the freezer, and equilibrate them at room temperature (18-28°C) for at least 30 minutes.**

**1. 1×PBST Wash Buffer:** Dilute 20×PBST Wash Buffer with deionized water in the volume ratio of 1:19(e.g. 1mL of 20×PBST Wash Buffer + 19mL of deionized water), which will be used to wash the plates.

**2. Detection Antibody:** Dilute Anti-T7 RNA Polymerase with Antibody Diluent Buffer in the volume ratio of 1:99(e.g. 1μL of Anti-T7 RNA Polymerase + 99μL of Antibody Diluent Buffer), which will be used to wash the plates.

**3. Streptavidin HRP Solution:** Dilute Streptavidin HRP with Enzyme Conjugate Diluent Buffer in the volume ratio of 1:99(e.g. 1μL of Streptavidin HRP + 99μL of Enzyme Conjugate Diluent Buffer), which will be used to wash the plates.

**4. Standards and samples were diluted with Sample Diluent Buffer.**

| No. | Concentration of Standards (ng/mL) | Volume of Standards (μL) | Volume of Sample Diluent Buffer (μL) | Totol Volume (μL) | Concentration (ng/mL) | Remaining volume (μL) |
|---|---|---|---|---|---|---|
| Pre-1 | 668000 | 5 | 395 | 400 | 8350 | 369.3 |
| 8 | 8350 | 30.7 | 969.3 | 1000 | 256 | 700 |
| 7 | 256 | 300 | 300 | 600 | 128 | 300 |
| 6 | 128 | 300 | 300 | 600 | 64 | 300 |
| 5 | 64 | 300 | 300 | 600 | 32 | 300 |
| 4 | 32 | 300 | 300 | 600 | 16 | 300 |
| 3 | 16 | 300 | 300 | 600 | 8 | 300 |
| 2 | 8 | 300 | 300 | 600 | 4 | 600 |
| 1 | / | / | 300 | 300 | 0 | 300 |

## Assay Procedure

**Note:**

Before use, mix all reagents thoroughly, and avoid bubbles.

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°C for later use.

**Procedure:**

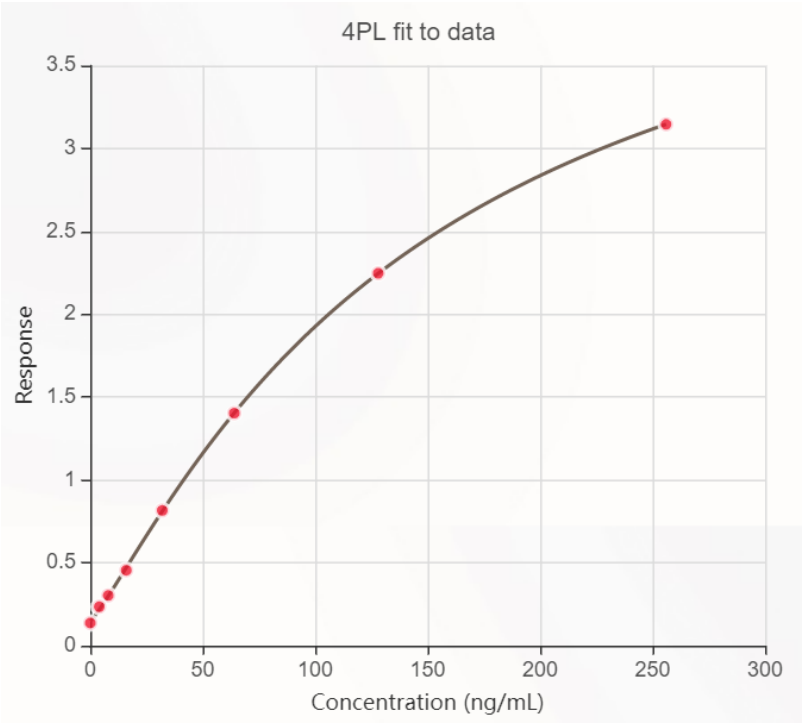
1. Add the 100 µl of T7 RNA Polymerase Standard, Sample Diluent Buffer, negative control into the corresponding wells of pre-coated microplate. Seal the plate with a sealing film and incubate it in a 37°C thermostatic incubator (200-300rpm) for 60 min.
2. Upon the completion of incubation, carefully remove the sealing film, and discard the liquid in the wells. Add at least 300 µl of 1×PBST Wash Buffer 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 each washing.
3. Add the 100 µl of Detection Antibody into wells of pre-coated microplate. Seal the plate with a sealing film and incubate it in a 37°C thermostatic incubator (200-300rpm) for 60 min.
4. Upon the completion of incubation, carefully remove the sealing film, and discard the liquid in the wells. Add at least 300 µl of 1×PBST Wash Buffer 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 each washing.
5. Add the 100 µl of Streptavidin HRP Solution into wells of pre-coated microplate. Seal the plate with a sealing film and incubate it in a 37°C thermostatic incubator (200-300rpm) for 60 min.
6. Upon the completion of incubation, carefully remove the sealing film, and discard the liquid in the wells. Add at least 300 µl of 1×PBST Wash Buffer 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 each washing.
7. Add the TMB substrate solution into the plate at 100 µl per well, gently mix well, seal the plate with a sealing film, and incubate it in a 25°C thermostatic incubator for 10 minutes (keep away from light)
8. Add the stop solution at 50 µl per well, gently mix well and then use a microplate reader to determine the absorbance per well at wavelength of 450 nm (reference 630nm).

## Calculation

Plot the calibration curve by four-parameter fitting.

## Typical Standard Curve

| Concentration of Standards (ng/mL) | OD(1) | OD(2) | Average |
|---|---|---|---|
| 256 | 3.109 | 3.183 | 3.146 |
| 128 | 2.281 | 2.213 | 2.247 |
| 64 | 1.411 | 1.394 | 1.4025 |
| 32 | 0.787 | 0.845 | 0.816 |
| 16 | 0.463 | 0.447 | 0.455 |
| 8 | 0.299 | 0.307 | 0.303 |
| 4 | 0.235 | 0.235 | 0.235 |
| 0 | 0.141 | 0.133 | 0.137 |




## Precision

CV%≤10%,RE%≤±15%

## Detection Limit

4- 256 ng/mL

LOD: 4ng/mL

LOQ: 2ng/mL

## Precautions

(1) If the sample to be tested is a purified product, it is usually recommended to test the original solution or dilute it by 2 times. For the first assay, at least 3 serial dilutions are recommended to produce at least one diluted sample within the range of the standard curve. Diluents should be mixed well before further analysis or dilution. Each sample was analyzed in duplicate to determine the correct T7 RNA polymerase residue value in the original sample.

(2) Reagents should be stored according to label instructions and equilibrated at room temperature before use.

(3)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°C 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.

(4) The volume of Streptavidin HRP, T7 RNA Polymerase Standard and Anti-T7 RNA Polymerase is very small. Before use, please centrifuge the tube briefly at high speed to allow the liquid on the tube wall or cap to settle to the bottom of the tube.

(5) Please use disposable tips during the experimental operation to avoid cross-contamination.

(6) Check the various reagents in the kit before use. Reagent dilution, sample addition and termination of reaction should be thoroughly mixed or shaken, which is particularly important for experimental results.

(7) During the washing process, the residual washing solution in the reaction well should be fully patted dry on a clean paper towel until the watermark is no longer visible. Do not put a paper towel directly into the reaction well to absorb water.

(8) The substrate chromogenic solution is sensitive to light, avoid prolonged exposure to light, and avoid contact with metals to affect the results.

(9) This product is a one-time use kit, please use it within the validity period.