T7 RNA Polymerase ELISA kit (Oscillation version)

Cat.No.: HYJ523

Product Description

This kit uses the double antibody sandwich method: The microwell plate has been pre-coated with anti-T7 RNA polymerase antibody. The sample is added and incubated with horseradish peroxidase (HRP) labeled antibody to form antibody-antigen-enzyme-labeled antibody complex. After washing, 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 depth of the color is positively correlated with the T7 RNA polymerase content in the sample. The absorbance (OD value) is measured at 450nm.

Components

Components	48T	96T
ELISA Microplate	8×6	8×12
Standard concentrate (0.1mg/ml)	0.025mL	0.05mL
Enzyme conjugate (100×)	0.03mL	0.06mL
Sample dilution buffer	15mL	30mL
Enzyme conjugate dilution buffer	3mL	6mL
Wash concentrate (10×)	15mL	30mL
Substrate solution	6mL	12mL
Stop solution	3mL	6mL
Plate sealer	2 pieces	4 pieces

Storage

Unopened kit should be stored at 2~8℃ for stability in shelf life.

1. Materials Required But Not Supplied

- Microplate reader with 450±10nm filter(better if can detect at 450 and 650 nm wavelength).
- Microplate shaker.

Instructions

Before You Begin

1. Bring all reagents to room temperature (23℃±2℃) before use.

2. Dilute concentrated washing liquid 10× with purified water according to 1:9 volume ratio into washing working liquid.
3. Dilute the Standard concentrate (0.1mg/ml) with Sample dilution buffer to 32ng/mL, 16ng/mL, 8ng/mL, 4ng/mL, 2ng/mL, 1ng/mL, 0.5ng/mL, 0ng/mL. We recommend standards can be diluted as following chart:

No.	Final Con. (pg/μL)	Dilution instruction	
		STE buffer	Standard
	1000	198uL	2μLStandard concentrate
A	32	387uL	12.8μL1000ng/mL
B	16	200uL	200μL solution A
C	8	200uL	200μL solution B
D	4	200uL	200μL solution C
E	2	200uL	200μL solution D
F	1	200uL	200μL solution E
G	0.5	200uL	200μL solution F
H	0	200uL	/

4. Dilute Enzyme conjugate (100×) to the working concentration by enzyme conjugate dilution buffer before use.

Using protocol

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. It is recommended that standard products and samples should be tested with multiple wells. First add 1X enzyme conjugate solution as per 50μL/ well, and then add the standard and sample as per 50μL/ well. * When the content of T7 RNA polymerase in the test sample cannot be determined, it is recommended to use the sample diluent to dilute the sample by multiple dilution (dilution 4 times, 8 times, 40 times, 200 times), and determine the dilution factor of the sample based on the value of the first test. If the dilution factor of the sample is determined, dilute the sample with sample diluent for testing. Ensure that the calculated values from the standard curve are within the linear range (0.25-32ng/ml).
3. Cover with the plate sealer. Incubate at 37℃ for 1hr with shaking at 500rpm.

4. Discard the liquid and pat it dry on a clean towel or absorbent paper. Fill each well with washing liquid (300μL) and let it stand for 15-30s. Shake off the washing liquid and pat it dry. Repeat washing the board three times.
5. Add 100μL of TMB substrate solution to each well. Cover with the plate sealer. Incubate at 37℃ for 15 min away from light.
6. Add 50μL of the Stop solution into each well. Read absorbance at 450/650nm .

Calculation of Results

1. Calculate the average light absorption value of the standard, blank control, and sample. The standard curve is drawn with the concentration of the standard product as the horizontal(X) coordinate and the average light absorption value of the standard product as the vertical(Y) coordinate. (The light absorption value in dual-wavelength detection mode is 450nm minus 650nm).
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 4 parameter non-linear fit model.

Performance

1. Lower limit of detection:< 0.1 ng/mL
2. Lower limit of quantitation: 0.25 ng/mL
3. Linearity: 0.25-32 ng/mL
4. Precision: Intra-assay CV ≤10%, Inter-assay CV ≤15%
5. Recovery: 80%~120%
6. Specificity: only specific recognition of T7 RNA polymerase, other IVT tool enzymes do not interfere with the measurements.

Notes

1. TMB reaction temperature and time are critical, please control them according to the instruction strictly.
2. During the washing process, the washing liquid should be soaked in the plate for 15~30s and then dried to fully wash the non-specific adsorbed components.
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(10x), warm to 37℃ and mix gently until the crystals are completely dissolved.

5. Avoid assay of samples containing sodium azide (NaN3), which will destroy the horseradish peroxidase activity and make the detection value low.
6. Different batches of reagents cannot be mixed. In addition, the pipette tips should not be mixed to avoid cross contamination.
7. For your safety and health, please wear a lab coat and wear gloves for experimental operation.
8. This product is intended for research use only, and shall not be used for clinical medical diagnosis and other irrational purpurposes.