Instructions for Use of T7 RNA Polymerase ELISA Detection Kit (2G)

The kit is intended for scientific research only and should not be used for diagnosis

Cat. No. TP001-2G

Product Introduction

This kit uses double-antibody sandwich enzyme-linked immunosorbent assay (ELISA) to add T7 RNA Polymerase standard and samples to be tested to the microtiter plate pre-coated with anti-T7 RNA Polymerase antibody, then add diluted biotin-labeled detection antibody and Streptavidin-HRP to form antibody + antigen + antibody-Biotin + SA-HRP complex, wash the plate and add TMB chromogenic solution for color development. TMB is converted from colorless to blue under the catalysis of HRP enzyme and finally to yellow under the action of stop solution. The shade of yellow was positively correlated with the amount of T7 RNA polymerase detected in the samples.

Assay range: 0.25~16ng/mL

Sensitivity: 0.012ng/mL

Specification

96T

Usage

It is applicable to the optimization of purification process of biological products, impurity control of intermediate process and release testing of final products.

Kit components

Components	Specification	Preparation
Standard	32 ng x 6 tubes	It is recommended to follow the dilution method
Coated Plate	8 wells × 12 strips	Ready-to-use
Sample Diluent Buffer	15 mL x 4 vials	Ready-to-use
Concentrated Wash Buffer (10x)	50 mL × 1 vial	Make a 10-fold dilution with deionized water.
Detection Antibody (100x)	65 µl × 1 tube	Make a 100-fold dilution with antibody diluent buffer.
Antibody Diluent Buffer	12 mL × 1 vial	Ready-to-use
TMB Chromogenic Solution	12 mL × 1 vial	Ready-to-use
Stop Solution	12 mL × 1 vial	Ready-to-use
Sealing Film	5 pieces	Ready-to-use
Instructions for Use	1 copy	Ready-to-use

Storage Conditions and Shelf Life

Sealed kits are valid for 12 months at 2-8 °C .

Materials to be self-prepared

- Microplate reader
- Micropipettes and tips
- New filter paper
- Thermostatic incubator
- Deionized water
- Vortex shaker

Pre-experiment Preparation

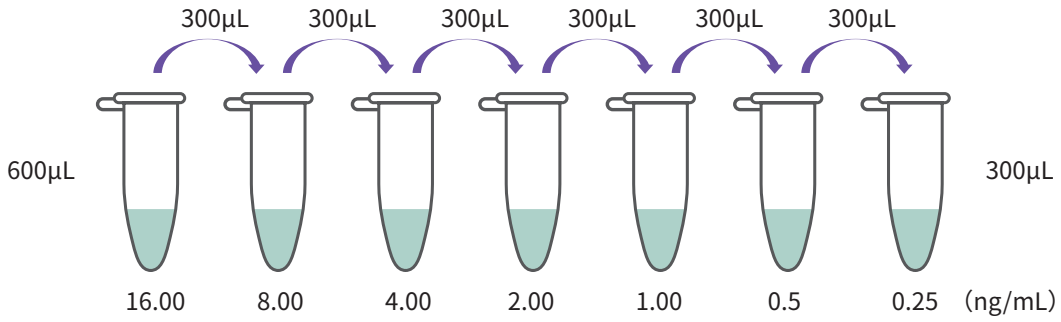
1. All reagents and samples to be tested should be restored to room temperature. All reagents are newly prepared and ready-to-use.

2. Preparation of 1x wash buffer: Equilibrate the concentrated wash buffer to room temperature, without crystallization. After mixing well, according to the amount used, dilute an appropriate amount of 10x wash buffer by 10 times with PBS at the ratio of 1: 9, to obtain 1x wash buffer.

3. Preparation of 1x detection antibody: Calculate the volume of working solution required for the test, dilute an appropriate amount of antibody diluent at the ratio of 1: 99, and mix well for later use.

4. Preparation of Standards:

Take a vial of standard, add 200 µL of deionized water, vortex and mix well, and obtain the final concentration of 160 ng/mL; Take 60 µL of standard solution with the concentration of 160 ng/ml, add to 540 µL of sample diluent buffer, vortex well to obtain a final concentration of 16 ng/ml. Pipette 300 µL of the sample diluent buffer to each centrifuge tube, and then perform 1: 1 gradient dilution according to the following figure.



Operation Procedures

All reagent components and samples to be tested should be restored to room temperature before use. Duplicate well assay is recommended for all standards and samples to be tested.

1. Mix all reagents thoroughly before use to avoid bubbles.
2. Determine the number of strips required according to the number of test wells, and place the remaining strips back in the aluminium foil bag and seal it.

3. Spiked and detection antibody working solution: Add 100 µL of standard, sample diluent working solution and negative control to each well. Add 50 µL detection antibody working solution into each well. After the plate is sealed with the sealing film, place it into a thermostatic shaker incubator for incubation at 37 °C and 600-800 rpm for 60 minutes.

4. Washing: Discard the liquid in each well, fill the wells with 1x PBST wash buffer (300 µL/well), allow to stand for 30 seconds and then discard the liquid in the wells; Repeat the above operations for 3 times and pat to dry on filter paper after each washing of the plate.

5. Chromogenic reaction: Add 100 µL of substrate chromogenic solution into each well, shake slightly to mix well, then seal the plate with microplate sealing film and hold it at 25 °C for 15 minutes for chromogenic reaction.

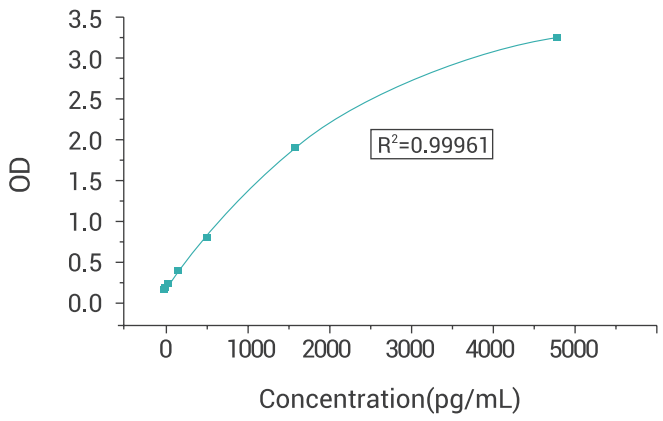
6. Assay: Add 100 µL of stop solution to each well and mix slightly. Select the main wavelength of 450 nm and reference wavelength of 630 nm of the microplate reader, and determine the absorbance (OD value) of each well.

Result processing

(1) OD processing of the standard curve (See the following example. For example only, please refer to the actual measurement for details)

Concentration of the standards (ng/mL)	OD1	OD2	Mean value
16	2.8708	2.9433	2.9071
8	2.0941	2.0731	2.0836
4	1.2382	1.2562	1.2472
2	0.7254	0.6866	0.7060
1	0.3880	0.3634	0.3757
0.5	0.2124	0.1877	0.2001
0.25	0.1233	0.117	0.1202
0	0.0393	0.0422	0.0408

(2) A standard curve is obtained by fitting the theoretical concentration of the standard and the corresponding OD value with four parameters (shown in the figure below).



Precautions

1. For the first detection of samples, it is recommended to perform at least three consecutive dilutions to produce at least one diluted sample within the range of the standard curve.
2. Reagents shall be stored according to label instructions and equilibrated at room temperature before use.
3. Before using, please equilibrate the coated plate to room temperature and then open the secondary package. The strips not used in the experiment shall be immediately placed back in the package for sealing and can be stored at 4 °C for one month. The remaining reagents shall be packaged or covered.
4. Please use disposable tips during experiments to avoid cross-contamination.
5. Check various reagents in the kit before use. Dilution, spiking and termination of the reaction with reagents shall be thoroughly mixed or shaken well, which is particularly important for the experimental results.
6. The residual wash buffer in the reaction wells during the washing process shall be patted thoroughly on a clean tissue until no water spots are visible. Do not place the tissues into the reaction wells to absorb water.
7. The substrate chromogenic solution is sensitive to light. Avoid prolonged exposure to light and avoid contact with metals that may affect the results.
8. This product is a disposable kit and shall be used within the validity period.

Disclaimer

Under all circumstances, the liability of our company for this product is only limited to the value of the product itself.

Jiangsu Hillgene Biopharma Co., Ltd.
A: Building 4, Yuewang Wisdom Valley, 1463 Wuzhong Avenue, Suzhou, Jiangsu Province, China
E. info@hillgene.com
W.www.bluekitbio.com / en.hillgene.com