Instructions for Use of Inorganic Pyrophosphatase ELISA Detection Kit

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

Cat. No. HG- IP001

Introduction

Inorganic pyrophosphatase (PPase) can catalyze the conversion of one molecule of pyrophosphate into two molecules of phosphate ions. PPase can avoid inhibiting the reaction system due to the accumulation of inorganic pyrophosphate in nucleic acid amplification experiments. In the production of mRNA vaccine products, PPase is added to increase yield. Therefore, detection of PPase residues is required for mRNA vaccine products.

This kit uses double-antibody sandwich enzyme-linked immunosorbent assay (ELISA) method. Add PPase standard and test samples to the microtiter plate precoated with anti-PPase antibody, then add diluted biotin-labeled PPase detection antibody, finally add streptavidin-HRP to form the 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 is positively correlated with the amount of PPase detected in the sample.

Assay range : 0.25 - 16 ng/mL

Limit of quantification : 0.25 ng/mL

Specification

96 T

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	lyophilized powder × 2 pieces	Gradient dilution with detection buffer
Coated Plate	8 wells × 12 strips	Ready-to-use
Diluent Buffer 1	45 mL x 1 vial	Ready-to-use
Diluent Buffer 2	30 mL x 1 vial	Ready-to-use
Wash Buffer (20x)	50 mL × 1vial	Make a 20-fold dilution with ultrapure water.
Detection Antibody (100x)	120 μL × 1 vial	Make a 100-fold dilution with Diluent Buffer 2.
Streptavidin-HRP (500x)	60 μL × 1 vial	Make a 500-fold dilution with Diluent Buffer 2.
TMB Substrate	15 mL × 1 vial	Ready-to-use
Stop Solution	10 mL × 1 vial	Ready-to-use
Sealing Film	5 pieces	Ready-to-use
Instructions for Use	1 copy	Ready-to-use

Storage and shelf life

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

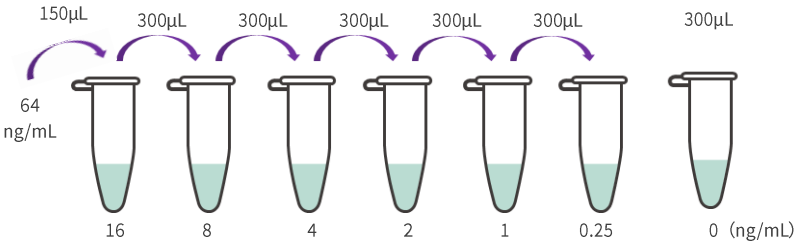
Apparatus and materials to be prepared by the user:

- Plate reader
- Thermostat plate shaker
- Micro pipette and tips
- Deionized water
- Unused filter paper
- 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 washing solution: Equilibrate the concentrated washing solution to room temperature, without crystallization. After mixing well, according to the usage volume, dilute 10x washing solution by 10 times with an appropriate amount of ultrapure water at the ratio of 1:9, to obtain 1x washing solution.
3. Preparation of 1x detection antibody: After the 100x detection antibody is fully dissolved, centrifuge and dilute the solution with Diluent 2 at the ratio of 1:99.
4. Preparation of 1x enzyme conjugate: After the 500x enzyme conjugate is fully dissolved, centrifuge and dilute the solution with Diluent 2 at a ratio of 1:499.
5. Preparation of standards

Prepare eight 1.5 mL centrifuge tubes and label them in turn according to the concentrations of the standards. Dissolve a vial of lyophilized standard with Diluent 1 according to the labeled amount, thoroughly dissolve and allow to stand for 10 minutes to obtain a solution concentration of 64 ng/mL. Add 450μL Diluent Buffer 1 to the first centrifuge tube, then add 300μL diluent 1 to the other centrifuge tubes, and take 150μL dissolved and mixed 64ng/mL standard product and add it into the first centrifuge tube to fully mix it to 16ng/mL, and then carry out 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. Preparation of reagents: Prepare all reagents to be tested, diluted standards and samples to be tested in advance.
2. Microplate strip determination: Calculate the microtiter strips required for the samples to be tested and standards, remove the microtiter strips from the aluminum foil bag, place the remaining microtiter strips back into the aluminum foil bag and seal the mouth of the bag, and store it at low temperature.
3. Soak the plate: Add 1×Wash Buffer (300μL/well) to soak the enzyme label plate, leave for 30 seconds, discard the liquid in the hole, and pat the plate dry. Washing the plate has an important impact on the test results, ensuring that there is no wash residue from the last clapping.
4. Sample incubation: Add the 100 μL of standards and the sample to be tested to each well, and ensure that the spot

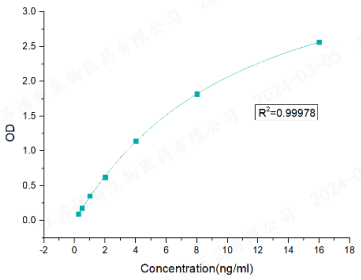
sampling is completed within 15 min, and incubate in a 37°C for 1 hour.
5. Plate washing: Discard the liquid in the wells, add 1 × washing solution (300 µL/well) to wash the plate for 5 times, and pat dry the residual liquid in the plate.
6. Detection Antibody incubation : Add the 100 µL of 1 × Detection Antibody to be tested to each well, and incubate in a 37°C for 1 hour.
7. Plate washing: Discard the liquid in the wells, add 1 × washing solution (300 µL/well) to wash the plate for 5 times, and pat dry the residual liquid in the plate.
8. Enzyme conjugate incubation: Add 1 × enzyme conjugate into plate with 100 µL/well, and incubate in a 37°C for 1 hour.
9. Plate washing: Discard the liquid in the wells, add 1 × washing solution (300 µL/well) to wash the plate for 5 times, and pat dry the residual liquid in ther plate.
10. Color development : the substrate solution was restored to room temperature 10 min before use, and add TMB Substrate into plate with 100 µL/well, and incubate at 37°C away from light for 15 min.
11. Termination: Add 50 µL stop solution into each well, and gently shake the plate until the color development is uniform.
12. Readings: Read the absorbance value at 450 nm/630 nm within 20 minutes. Take 450 nm as detection wavelength and 630 nm as reference wavelength.

Results processing

1. OD processing of the standard curve (See the following example, which is only for example purpose. Please refer to the actual measurement for details):

Standard concentration (ng/mL)	OD value (1)	OD value (2)	Mean value
16	2.698	2.612	2.655
8	1.952	1.864	1.908
4	1.202	1.265	1.234
2	0.699	0.729	0.714
1	0.439	0.454	0.447
0.5	0.265	0.281	0.273
0.25	0.192	0.183	0.188
0	0.094	0.091	0.093

2. The standard curve is obtained by 4-parameter fitting with the theoretical standard concentrations and the corresponding OD values (as shown in the figure below).



Precautions

1. When the sample is tested for the first time, it is recommended to perform dilution with at least 3 consecutive dilution factors, so as to generate at least one diluted sample within the range of the standard curve.
2. The reagents should be stored as indicated on the label, and should be equilibrated to room temperature before use.
3. Before using the coated microtiter plates, please equilibrate to room temperature and then open the secondary packaging. The strip plates not used in the test should be immediately placed back into the package and sealed properly, and can be stored at 4℃ for one month. Other unused reagents should be packaged or covered properly.
4. Please use disposable tips during experimental operation to avoid cross contamination.
5. Please check each individual reagent in the kit fully before use. To obtain accurate assay results, it is of special importance to mix well or shake well the reagents for dilution, loading, and reaction termination.
6. When washing residual Wash Buffer in the reaction wells, pat the plate dry adequately on clean tissue papers until watermark is no longer visible. Do not put the tissue paper into the well for liquid absorption.
7. The TMB Substrate is photosensitive, thus long-time exposure to illumination should be avoided; avoid contact with metal, otherwise, the assay results may be affected.
8. The kit is intended for single use. Please use within the shelf life.

Disclaimer

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

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