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Pyrophosphatase, Inorganic ELISA Kit

Product Number: PA101

Shipping and Storage

1. Please store the PPase Standard in a dry and dark place at -20±5℃, and store the remaining components in a dark place at 2-8℃. The shelf life is one year;
2. After opening the reagent kit, please use it as soon as possible.

Components

Component	PA101
PPase Microplate	96T
PPase Detection Antibody	150μl
PPase Standard	0.15ml
20×Wash Buffer	30ml
Dilution Buffer 1	30ml
Dilution Buffer 2	15ml
Color Reagent A	6ml
Color Reagent B	6ml
Stop Reagent	6ml
Sealing Film	2

Note: 1) PPase Detection Antibody: HRP labeling, dilution ratio 1:100
2)Different batches of components cannot be mixed.

Description

Inorganic pyrophosphatase (PPase) can catalyze the hydrolysis of inorganic pyrophosphate to produce orthophosphate: $P_2O_7^{4-}+H_2O+PPase \rightarrow 2HPO_4^{2-}$. In nucleic acid amplification experiments, PPase can hydrolyze the inorganic pyrophosphate generated during the reaction, avoiding its inhibition on the reaction system. The removal of pyrophosphate can shift the reaction equilibrium towards the product generation end.

In the production process of mRNA vaccine drugs, inorganic pyrophosphatase can increase the generation of mRNA products. According to regulations, residual testing of mRNA vaccine stock solution is required. Among them, PPase, as a protein product, belongs to the category of protein residue testing, so quantitative testing of its residual amount is required.

This reagent kit uses double antibody sandwich enzyme-linked immunosorbent assay (ELISA) to detect PPase, which can detect and quantitatively analyze PPase residues in mRNA stock with high sensitivity and specificity.

Principle

This reagent kit adopts a dual antibody sandwich ELISA method, pre coated with PPase capture antibodies. After adding the sample, the sample is captured to form an antibody antigen complex, and then HRP labeled PPase detection antibodies are added to form an antibody antigen antibody "sandwich" complex. Finally, TMB was added for color development. After the reaction was terminated, the absorbance value (OD value) was read at a wavelength of 450nm/630nm. The PPase content in the sample was positively correlated with the OD value.

Note

1. Before the experiment, the experimental reagents should be equilibrated to room temperature according to relevant requirements.
2. All reagents should be thoroughly mixed before use to ensure their uniformity and accuracy.

3. Strictly control the reaction temperature and time.
4. The measured value cannot exceed the normal range.
5. The number of times to wash the board, the amount of washing liquid, the soaking time of the washing liquid, and the strength of the washing board are all issues that should be paid attention to.
6. Try to avoid bubbles in the micropores when terminating the reaction.
7. Each experiment requires the preparation of corresponding standard curves, and different reagent kits and standard curves from different days cannot be mixed.
8. Pay attention to timely replacing the sampling tank and gun head between different samples and steps to avoid cross contamination.
9. The reading of light absorption value should be completed within 20 minutes after adding the termination solution.
10. High concentration during color rendering may produce black flocculent substances, which is a normal phenomenon and does not affect the final reading result to a slight extent.

Protocol

1. Preparation work

All reagent components and test samples need to be restored to room temperature before use.

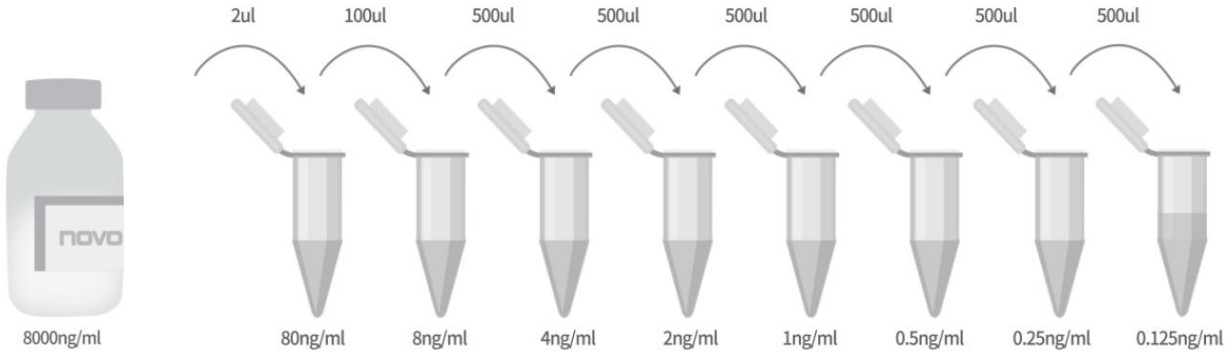
1.1. Preparation of 1×Wash Buffer: Equilibrate the 20×Wash Buffer to room temperature until the crystals are completely dissolved, mix well, and dilute it 20 times with deionized water or ultrapure water. The amount of 1×Wash Buffer used per well after dilution is 1.5ml, and it can be stably stored for 1 week at 2-8℃.

1.2. Dilution of PPase Detection Antibody: Mix well before use, and then dilute PPase Detection Antibody with Dilution Buffer 2 in a 1:100 dilution ratio.

1.3. Preparation of substrate solution: Mix Color Reagent A and Color Reagent B in a 1:1 ratio, prepare and use as needed. 100μl per hole. If the mixed substrate turns blue, do not use.

1.4. Dilution of PPase Standard: Take 2μl of PPase Standard (8000ng/ml) into a new EP tube, add 198μl Dilution Buffer 1 to dilute to a concentration of 80ng/ml, then take 100μl of the diluted sample (80ng/ml) into a new EP tube, add 900μl Dilution Buffer 1 to dilute to a concentration of 8ng/ml, as the first point on the standard curve. Take another 500μl of 8ng/ml concentration sample and transfer it to a new EP tube. Add 500μl of Dilution Buffer and perform a series of 2-fold gradient dilutions to obtain concentrations of 4, 2, 1, 0.5, 0.25, and 0.125ng/ml. Each experiment requires the preparation of corresponding standard curves, and different reagent kits and standard curves of different orders cannot be mixed.

Note: After re dissolving the standard product, please pack it separately and store it at -20℃.



2. Protocol

All operations should be carried out at room temperature. It is recommended to perform dual well assays on both the standard sample and the test sample.

2.1. Incubation of samples and test antibodies: Add the diluted PPase Standard and the test sample to the enzyme-linked immunosorbent assay (ELISA) plate, 100μl/well, respectively; Add the diluted PPase Detection Antibody to the enzyme-linked immunosorbent assay plate at a rate of 100μl/well, mix well, cover with a sealing membrane, and incubate at 37℃ for 2 hours.

2.2. Washing plate: Discard the liquid in the hole, wash the plate with 1×Wash Buffer at a rate of 300μl/well, wash the plate 5 times, and pat dry.

2.3. Color development: Add the prepared substrate solution to the enzyme-linked immunosorbent assay (ELISA) plate at a rate of 100μl/well, mix well, cover with a sealing film, and incubate at 37°C in the dark for 20 minutes.

2.4. Termination: Add Stop Reagent to the enzyme-linked immunosorbent assay (ELISA) plate at a rate of 50μl per well. Gently shake the ELISA plate until the color is uniform, ensuring that the color development time for each well is the same.

2.5. Reading value: Place the enzyme-linked immunosorbent assay (ELISA) plate into the ELISA reader. It is recommended to perform a dual wavelength test (test wavelength 450nm, reference wavelength 630nm) to read the absorbance, and read the value within 20 minutes.

3. Concise operation process

Add 100μl standard/sample to each well

⬇

Add 100μl of diluted PPase Detection Antibody to each well

⬇ Incubate at 37°C for 2 hours

Wash the plate 5 times with a 1xWash Buffer at a rate of 300μl/well, and pat dry the liquid in the well

⬇

Add 100μl of substrate solution to each well and mix well

⬇ Incubate at 37°C in dark for 20 minutes

Add 50μl stop Reagent to each well and mix well, then check the absorbance value

4. Data processing

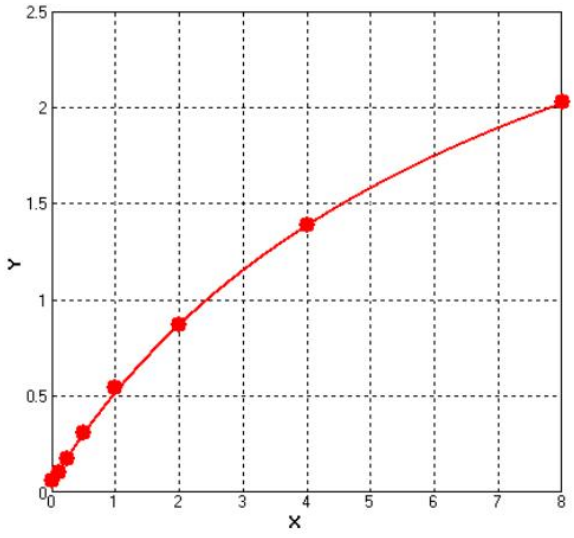
If the OD value of the test sample exceeds the OD value at the highest point of the standard curve, the sample needs to be diluted and retested.

Draw a standard curve with the standard concentration as the x-axis and the standard absorbance value as the y-axis. It is recommended to use ELISACalcexe regression and fitting calculation software for four parameter fitting to calculate the concentration of PPase in the sample.

Example display:

The following standard curve chart is for reference only, and the sample content should be calculated based on the standard curve drawn for the same experimental standard.

ng/ml	OD450
8	2.0245
4	1.3855
2	0.8690
1	0.5440
0.5	0.3105
0.25	0.1715
0.125	0.1060
0	0.0585
R2	0.99



Product performance indicators

1. Sensitivity

The sensitivity of the reagent kit detection is 0.125ng/ml

2. Blank limit

Blank limit of reagent kit ≤ 0.1

Parallel hole(n=16)	Numerical value
AVE	0.0613
SD	0.0072
AVE+2SD≤0.1	0.0757

3. Detection scope

The detection range of the reagent kit is 0.125-8ng/ml

4. Recovery rate of spiking

Recovery rate of reagent kit spiking: 80% -120%

Theoretical concentration (ng/ml)	Calculate the average concentration back(ng/ml)	Average spiked recovery rate
4	4.215	105%
1	0.982	98%
0.25	0.255	102%

5. Precision

The intra batch precision of the reagent kit is ≤ 15%.

Theoretical concentration(ng/ml)	Sample number	Calculate the average concentration back(ng/ml)	SD	CV
4	10	4.626	0.430	9.30%
1	10	1.036	0.066	6.43%
0.25	10	0.273	0.020	7.40%

Common problems and solutions in ELISA

1. High background

1.1. Contamination of substrate solution: The substrate solution itself is colorless, ensuring that the substrate is not contaminated with metal ions or oxidants before use and stored in a dark place.

1.2. PPase Detection Antibody Dilution Error: Ensure that PPase Detection Antibody is diluted according to the specified requirements in the instruction manual. A high concentration of PPase Detection Antibody may cause a high background.

1.3. Incorrect washing: Insufficient or missed washing can lead to a high background. Verify the amount of ELISA wash solution and ensure that all washing steps are completed, allowing the wash solution to remain in the well for at least 15-30 seconds. After each wash, ensure that there is no residue.

1.4. The incubation time and temperature are incorrect: The incubation time and temperature are strictly in accordance with the ELISA operating procedures.

1.5. Consumable contamination: Use clean pipes, gun heads, and other consumables.

1.6. Dilution contaminated: Most ELISA diluents are protein matrices, and it is best to prepare and use them immediately.

1.7. Sample contamination: Avoid cross contamination of samples.

1.8. Sample concentration too high: Determine the appropriate dilution ratio.

2. High CV value inside the board

2.1. Damage to the coating layer during washing/suction: When washing or adding standards and samples, be careful not to scratch the inside of the microplate.

2.2. Sample non-uniformity: Mix the sample evenly before adding to ensure that the sample is uniform.

2.3. Edge effect: Avoid incubating the plate under conditions with significant temperature changes (such as ventilation or windows), and add PBS of the same volume as the test sample to the outermost edge of the enzyme-linked immunosorbent assay plate. Do not stack the plates together during incubation.

2.4. Incorrect washing: Insufficient or missed washing can lead to an increase in CV value. Do not reduce the volume of washing solution or washing steps, let the washing solution stay in the microplate for 15-30 seconds.

3. Inaccurate standard curve

3.1. Standard dilution error: Concentrated standard is continuously diluted and mixed correctly.

3.2. Standard storage issue: Standard and diluent should be stored according to the instructions.

3.3. Measurement issue: Bubbles in the hole cause abnormal readings. When terminating the reaction, try to avoid bubbles in the micropores.

4. No signal or weak color display

4.1. Reagent storage issue: The reagent has expired, the enzyme-linked antibody is inactivated or its potency is reduced, and different batches of diluents are used interchangeably. The pH value of the solution is incorrect: Generally, the pH value of the diluent should be maintained between 7.2 and 7.4.

4.2. Operational issue: Misaddition or omission of relevant reagents, reagents not equilibrated to room temperature before use, incubation time and incubation temperature not meeting experimental requirements.

5. The measured value exceeds the normal range

5.1. Dilution issue: Dilute the sample and standard according to the operating procedure, using the diluent provided by the reagent kit.

5.2. Measurement issue: Verify the detection wavelength provided in the manual to ensure that the wavelength is correct.

5.3. Color development time: The time displayed in the operation process may need to be changed according to temperature or other experimental conditions (temperature, Kit shelf life, humidity). It is recommended to add a termination solution when the highest concentration standard hole appears dark blue.

5.4. Incubation issue: The incubation time and temperature are strictly in accordance with the ELISA operating procedures. During the incubation period, the cover plate can prevent liquid evaporation and contamination.