Inorganic Pyrophosphatase ELISA Kit

Cat.No.: HYJ528

Product Description

This kit employs the principle of the double antibody sandwich method for the quantitative detection of inorganic pyrophosphatase concentrations in samples. The microplate is pre-coated with anti-inorganic pyrophosphatase antibodies. Inorganic pyrophosphatase present in the sample binds to the antibodies immobilized on the wells. Subsequently, a horseradish peroxidase (HRP)-labeled antibody is added, which specifically binds to the inorganic pyrophosphatase in the sample, forming an antibody-antigen-enzyme-labeled antibody complex. After adding the TMB substrate solution, HRP catalyzes the substrate to produce a blue product, which changes to yellow upon addition of an acidic stop solution. The intensity of the yellow coloration is directly proportional to the concentration of inorganic pyrophosphatase in the sample. The absorbance (OD value) is measured at 450 nm using a microplate reader, and the concentration of inorganic pyrophosphatase in the sample is calculated based on a standard curve.

Components

Components	48T	96T
ELISA Microplate	8×6	8×12
Enzyme conjugate (100×)	60uL	120uL
Dilution buffe	15mL	30mL
TMB substrate solution	6mL	12mL
Stop solution	3mL	6mL
Concentrated wash buffer (10x)	15mL	30mL
Standard (0.1mg/mL)	25uL	50uL
Plate sealer	2 pieces	4 pieces

Storage

1. The standard (0.1 mg/mL) in the kit should be stored at -25~-15℃, while the remaining components must be stored at 2~8℃. To ensure storage stability, avoid exposure to strong light. The shelf life is 12 months.

2. For used kit: Once the microplate is opened, please cover unused wells with plate sealer and return to the foil pouch containing the desiccant pack, zip-seal the foil pouch and return to 2~8℃ as soon as possible after use. Other reagents should be returned to 2~8℃ as soon as possible after use.

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 kit components and samples to room temperature (18-25℃) before use. If the kit will not be used up in one time, please only take out strips and reagents for present experiment, and leave the remaining strips and reagents in required condition.
2. Wash buffer: dilute 30mL of 10×concentrated wash buffer with 270mL of deionized or distilled water to prepare 300mL of 1× wash buffer.
3. Standard: Dilute the Standard concentrate (0.1mg/ml) with dilution buffer to 4ng/mL, 2ng/mL, 1ng/mL, 0.5ng/mL, 0.25ng/mL, 0.125ng/mL, 0.0625ng/mL, 0ng/mL. We recommend standards can be diluted as following chart:

No.	Final Con. (pg/μL)	Dilution instruction	
		STE buffer	Standard
	1000	198uL	2μL 0.1mg/mL standard
A	4	498uL	2μL1000ng/mL
B	2	250uL	250μL solution A
C	1	250uL	250μL solution B
D	0.5	250uL	250μL solution C
E	0.25	250uL	250μL solution D
F	0.125	250uL	250μL solution E
G	0.0625	250uL	250μL solution F
H	0	250uL	/

4. Enzyme conjugate working solution: briefly spin or centrifuge the stock solution before use. Dilute them to the working concentration with dilution buffer.

5. TMB substate: aspirate the needed dosage of the solution with sterilized tips and do not dump the residual solution into the vial again. TMB substate is sensitive to light, don't exposure TMB substrate to light for a long time.

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. Add 100μL each of dilutions of standard, blank and samples into the appropriate wells. Cover with the plate sealer. Incubate for 1hr at room temperature with shaking at 500rpm. * When the content of inorganic pyrophosphatase in the test sample cannot be determined, it is recommended to use the diluent buffer to dilute the sample by multiple dilution 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 diluent buffer for testing. Ensure that the calculated values from the standard curve are within the linear range (0.0625-4ng/ml).
3. Wash step: Aspirate the solution and wash with 300μL wash buffer to each well and let it stand for 15-30s. Discard wash buffer completely by snapping the plate onto absorbent paper. Totally wash 3 times.
4. Add 100μL of Enzyme conjugate working solution into each well. Cover with the plate sealer. Incubate for 1hr at room temperature with shaking at 500rpm.
5. Repeat wash step.
6. Add 100μL of TMB substrate solution into each well. Cover with the plate sealer. Incubate at 37°C in the dark for 15 minutes. The liquid will turn blue by the addition of substrate solution.
7. Add 50μL of stop solution into each well. The liquid will turn yellow by the addition of stop solution. Then run the microplate reader and conduct measurement at 450nm immediately.

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: 0.0625 ng/mL
3. Linearity: 0.0625~4 ng/mL
4. Precision: Intra-assay CV ≤10%, Inter-assay CV ≤15%
5. Recovery: 80%~120%
6. Specificity: only specific recognition of inorganic pyrophosphatase, 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 orreagents 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 purposes.