Pyrophosphate Colorimetric Microplate Assay Kit

Instructions for Use | Cat. No.: ARS6907

Detection and Quantification of Pyrophosphate (PPi) Content in Serum, Plasma, Tissue extracts, Cell lysate, Cell culture media and Other biological fluids Samples.

For research use only. Not for diagnostic or therapeutic procedures.

1. Introduction

Inorganic pyrophosphate (diphosphate, pyrophosphoric acid or PPi) is a key intermediate in energy metabolism and substance synthesis within living organisms, directly participating in the synthesis of various core biomolecules such as ATP, nucleic acids, and coenzymes. Simultaneously, as a crucial regulatory factor, it not only inhibits ectopic calcification to maintain skeletal health but its abnormal deposition can also lead to pathological conditions like pseudogout. The metabolic balance of pyrophosphate is vital for life processes.

Pyrophosphate Colorimetric Microplate Assay Kit provides a simple and direct procedure for measuring pyrophosphate content in a variety of samples. In this assay, pyrophosphate is enzymatically detected through a series of reactions which utilize a proprietary enzyme mix and substrates mix, generating a stable product. The rate of increase in the absorbency at 550 nm, which is proportional to pyrophosphate content.

2. Kit Components

Component	Volume	Storage
96-Well Microplate	1 plate	-
Assay Buffer	30 ml x 4	4 °C
Diluent A	10 ml x 1	4 °C
Diluent B	10 ml x 1	4 °C
Enzyme Diluent	1 ml x 1	4 °C
Reaction Buffer	Powder x 1	4 °C, keep in dark
Substrate	Powder x 1	4 °C, keep in dark
Enzyme	Powder x 1	-20 °C, keep in dark
Dye Reagent	Powder x 1	4 °C, keep in dark
Standard	Powder x 1	4 °C
Plate Adhesive Strips	3 Strips	-
Technical Manual	1 Manual	-

3. Materials Required But Not Provided

1. Microplate reader to read absorbance at 550 nm
2. Distilled water
3. Pipettor, multi-channel pipettor
4. Pipette tips
5. Mortar
6. Centrifuge
7. Timer
8. Convection oven

4. Reagent Preparation

Reaction Buffer: Add 10 ml Diluent A to dissolve before use. Keep in dark and store at -20 °C for 1 week.

Substrate: Briefly centrifuge prior to opening. Add 1 ml Diluent B to dissolve before use. Keep in dark and store at 4 °C for 1 week or -20 °C for 1-2 months.

Standard: Briefly centrifuge prior to opening. Dissolve in 1 ml Diluent B to generate 20 mmol/L stock standard solution, store at 4 °C for 1 month after reconstitution. Dilute to 2 mmol/L top standard solution by adding 0.1 ml stock solution into 0.9 ml Diluent B. Perform 2-fold serial dilutions of the top standard solution using Diluent B to make the standard curve. The concentration of standard curve could be 2000/1000/500/250/100/50 μmol/L.

Dye Reagent: Add 6 ml distilled water to dissolve before use. Keep in dark and store at 4 °C for 1-2 weeks or -20 °C for 2-3 months.

Enzyme: Briefly centrifuge prior to opening. Add 1 ml Enzyme Diluent to dissolve before use. Keep in dark and store at -20 °C for 2-3 months.

5. Sample Preparation

1. For cell samples

Collect cell or bacteria into centrifuge tube, discard the supernatant after centrifugation, add 1 ml Assay buffer for 5×10^6 cell or bacteria, sonicate (with power 20%, sonicate 3s, interval 10s, repeat 30 times); centrifuged at 10000g 4 °C for 10 minutes, take the supernatant into a new centrifuge tube for detection.

2. For tissue samples

Weigh out 0.1 g tissue, homogenize with 1 ml Assay buffer, centrifuged at 10000g 4 °C for 10 minutes, take the supernatant into a new centrifuge tube for detection.

3. For liquid samples

Detect directly.

6. Assay Procedure

Add following reagents into the microplate:

Reagent*	Sample**	Standard	Blank
Reaction Buffer	100 μl	100 μl	100 μl
Distilled water	--	--	20 μl
Sample	20 μl	--	--
Standard	--	20 μl	--
Substrate	10 μl	10 μl	10 μl
Enzyme	10 μl	10 μl	10 μl
Dye Reagent	60 μl	60 μl	60 μl

Mix, put it into the convection oven, 45 °C for 45 minutes; record absorbance measured at 550 nm.

Note:

*Reagents must be added sequentially and should not be premixed prior to addition.

**The concentrations can vary over a wide range depending on the different samples. For unknown samples, we recommend doing a pilot experiment & testing several doses to ensure the readings are within the standard curve range.

**For colored samples, we recommend setting a parallel sample background control well with same volume of sample only. Other reagents were replaced by distilled water to the same total volume. Subtract the OD value of the sample background control from the OD value of the sample to correct for interference from the sample's own color.

7. Calculation

1. Calculate the sample concentration in ASSAY PROCEDURE according to the slope of the standard curve

$$C = \frac{(OD_{Sample} - OD_{Blank}) - Intercept}{Slope} \times n \quad (mmol/L)$$

Calculate the initial concentration according to sample preparation procedure.

2. According to one point of the standard OD and concentration

2.1 According to the quantity of cells

$$C = \frac{(C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Blank})}{(OD_{Standard} - OD_{Blank}) \times N \times (V_{Sample} / V_{Assay})} \quad (mmol/10^4)$$

2.2 According to the weight of sample

$$C = \frac{(C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Blank})}{(OD_{Standard} - OD_{Blank}) \times W \times (V_{Sample} / V_{Assay})} \quad (mmol/g)$$

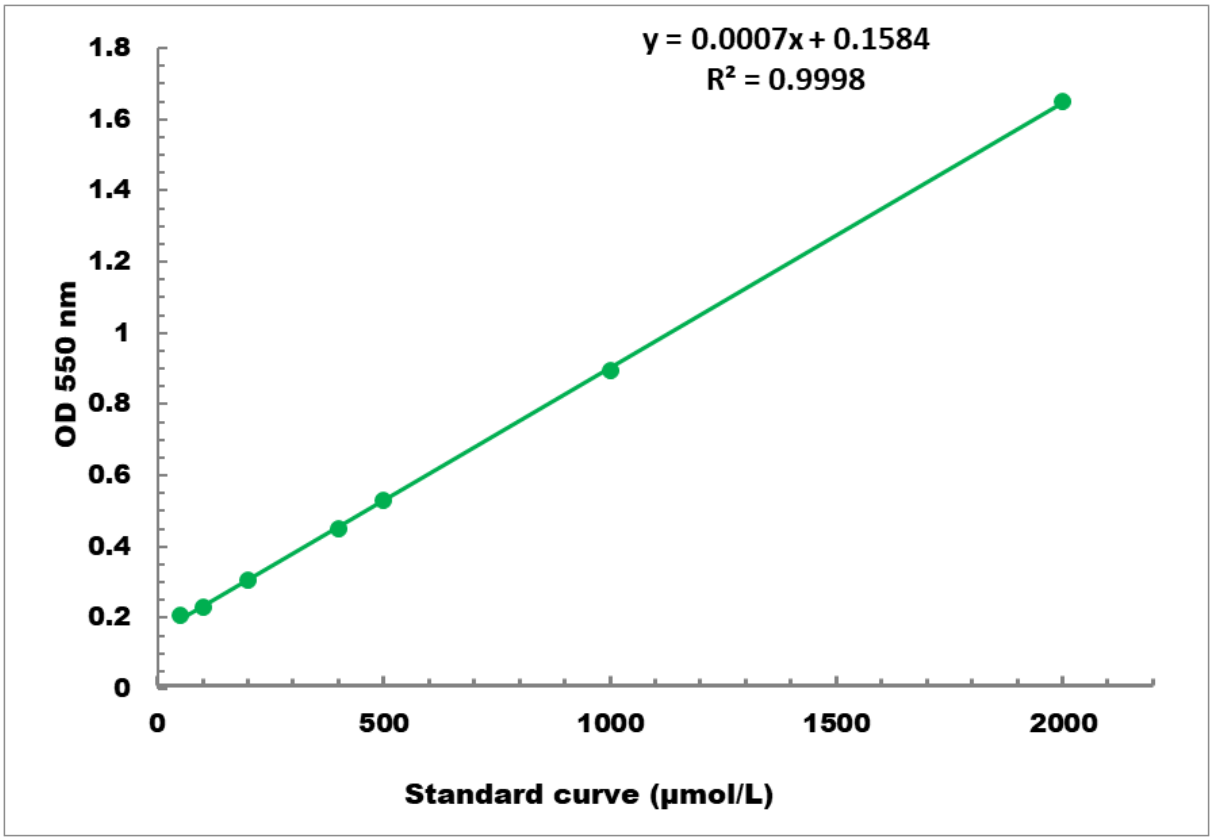
2.3 According to the volume of sample

$$C = \frac{(C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Blank})}{(OD_{Standard} - OD_{Blank}) \times V_{Sample}} \quad (mmol/ml)$$

Item	Definition
Slope	the absorbance slope of standard curve
n	the dilution factor
$C_{Standard}$	the standard concentration, mg/ml
$V_{Standard}$	the volume of standard in assay procedure, μl
V_{Sample}	the volume of sample in assay procedure, μl
V_{Assay}	the volume of Assay Buffer, μl
W	the weight of sample, g
V	the volume of sample in sample preparation, ml
N	the quantity of cell, 10^4

8. Typical Data

The standard curve is for demonstration only. A standard curve must be run with each assay.



Detection Range: 50 μmol/L - 2000 μmol/L