PhosphoWorks™ Fluorimetric Pyrophosphate Assay Kit *Enhanced Selectivity*

Catalog number: 21614
Unit size: 200 Tests

Component	Storage	Amount
Component A: Assay Buffer	Freeze (<-15 °C)	1 bottle (25 mL)
Component B: PPi Sensor	Freeze (<-15 °C), Minimize light exposure	1 vial (lyophilized powder)
Component C: Pyrophosphate Standard	Freeze (<-15 °C), Minimize light exposure	1 mL (50 mM)
Component D: DMSO	Freeze (<-15 °C)	1 vial (100 µL)

OVERVIEW

Pyrophosphate (PPi) is produced by a number of biochemical reactions, such as ATP hydrolysis, DNA and RNA polymerizations, cyclic AMP formation by the enzyme adenylate cyclase and the enzymatic activation of fatty acids to form their coenzyme A esters. Our PhosphoWroks™ Pyrophosphate Assay Kit provides the most robust spectrophotometric method for measuring pyrophosphate. This kit uses our proprietary fluorogenic pyrophosphate sensor that has its fluorescence intensity proportionally dependent upon the concentration of pyrophosphate. The PPi sensor used in the kit has quite high selectivity to PPi compared to phosphate and ATP. Our assay is much easier and more robust than the enzyme-coupling pyrophosphate methods that require at least two enzymes for their pyrophosphate detections. The kit provides all the essential components for assaying pyrophosphate. This kit has been successfully used in high throughput screening (HTS). Please inquire special HTS bulk package discount for the screening of >10,000 assays.

AT A GLANCE

Protocol summary

1. Prepare PPi Sensor working solution (50 µL)
2. Add Pyrophosphate standards and/or test samples (50 µL)
3. Incubate at room temperature for 10 to 30 minutes
4. Monitor fluorescence intensity at Ex/Em = 370/470 nm (Cutoff = 455 nm)

Important Thaw all the kit components at room temperature before starting the experiment.

KEY PARAMETERS

Instrument:	Fluorescence microplate reader
Excitation:	370 nm
Emission:	470 nm
Cutoff:	455 nm
Recommended plate:	Solid black

PREPARATION OF STOCK SOLUTIONS

Unless otherwise noted, all unused stock solutions should be divided into single-use aliquots and stored at -20 °C after preparation. Avoid repeated freeze-thaw cycles.

1. PPi Sensor stock solution (200X):
Add 50 µL of DMSO (Component D) into the vial of PPi Sensor (Component B) to make 200X PPi Sensor stock solution.

Note 25 µL of the PPi Sensor stock solution is enough for one 96-well plate.

PREPARATION OF STANDARD SOLUTION

Pyrophosphate standard

For convenience, use the Serial Dilution Planner:
https://www.aatbio.com/tools/serial-dilution/21614

Prepare 1 mM Pyrophosphate standard by adding 10 µL of 50 mM Pyrophosphate Standard (Component C) into 490 µL of Assay Buffer (Component A), or buffer of your choice (preferably 50 mM Hepes buffer, pH 7) to make 1 mM Pyrophosphate standard. Take 1 mM Pyrophosphate standard (PS7) to perform 1:3 serial dilutions to get serially diluted Pyrophosphate standards (PS6-PS1) with Assay Buffer (Component A).

PREPARATION OF WORKING SOLUTION

Add 25 µL of 200X PPi Sensor stock solution to 5 mL of Assay Buffer (Component A), and mix them well.

Note Due to the high sensitivity of this assay to PPi, it is important to use PPi-free labware and reagents.

Note DTT (1 uM) will increase the background, MgCl2 ≥ 2mM decrease the response.

SAMPLE EXPERIMENTAL PROTOCOL

Table 1. Layout of pyrophosphate standards and test samples in a solid black 96-well microplate. PS = Pyrophosphate Standard, BL = Blank Control, TS = Test Sample.

BL	BL	TS	TS
PS1	PS1	...	...
PS2	PS2	...	...
PS3	PS3		
PS4	PS4		
PS5	PS5		
PS6	PS6		
PS7	PS7		

Table 2. Reagent composition for each well

Well	Volume	Reagent
PS1-PS7	50 µL	Serial Dilution (1 to 1000 µM)
BL	50 µL	Assay Buffer (Component A)
TS	50 µL	Sample

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1. Prepare Pyrophosphate standards (PS), blank controls (BL), and test samples (TS) according to the layout provided in Tables 1 and 2. For a 384-well plate, use 25 µL of reagent per well instead of 50 µL.

2. Add 50 µL of PPi Sensor working solution to each well of Pyrophosphate standard, blank control, and test samples to make the total assay volume of 100 µL/well. For a 384-well plate, add 25 µL of PPi Sensor working solution into each well instead, for a total volume of 50 µL/well.

3. Incubate the reaction at room temperature for 10 - 30 minutes, protected from light.

4. Monitor the fluorescence increase with a fluorescence plate reader at Ex/Em = 370/470 nm (Cutoff = 455 nm)

EXAMPLE DATA ANALYSIS AND FIGURES

The reading (RFU) obtained from the blank standard well is used as a negative control. Subtract this value from the other standards' readings to obtain the base-line corrected values. Then, plot the standards' readings to obtain a standard curve and equation. This equation can be used to calculate PPi samples. We recommend using the Online Linear Regression Calculator which can be found at:

https://www.aatbio.com/tools/linear-logarithmic-semi-log-regression-online-calculator

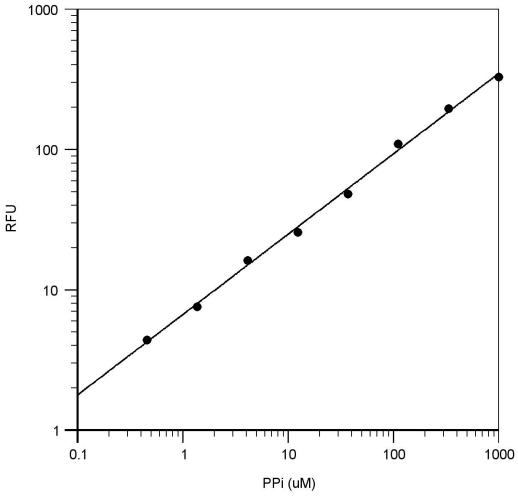

Figure 1. Pyrophosphate, ATP and phosphate dose responses were measured with PhosphoWorks™ Fluoremetric Pyrophosphate Assay Kit in a solid black 96-well plate using a fluorescence microplate reader.

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