PhosphoWorks™ Fluorimetric Pyrophosphate Assay Kit *Blue Fluorescence*

Catalog number: 21611
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), Dessicated, Avoid Light	1 vial (lyophilized powder)
Component C: Pyrophosphate Standard	Freeze (<-15 °C), Avoid Light	1 mL (50 mM)
Component D: DMSO	Freeze (<-15 °C)	1 vial (200 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. 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 Pyrophosphate standards and/or test samples (50 µL)
2. Add Pyrophosphate working solution (50 µL)
3. Incubate at room temperature for 10 to 30 minutes
4. Monitor fluorescence intensity at Ex/Em = 316/456 nm (Cutoff = 420 nm)

Important Thaw all the four components at room temperature before use.

KEY PARAMETERS

Instrument:	Fluorescence microplate reader
Excitation:	316 nm
Emission:	456 nm
Cutoff:	420 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. Protect from light.

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

2. Pyrophosphate standard solution (1 mM):
Add 10 µL of 50 mM Pyrophosphate Standard (Component C) into 490 µL of ddH_2O or 50 mM Hepes buffer (pH 7) to make 1 mM Pyrophosphate standard solution.

PREPARATION OF STANDARD SOLUTION

Pyrophosphate standard

For convenience, use the Serial Dilution Planner:
https://www.aatbio.com/tools/serial-dilution/21611

Add 50 µL of 1 mM Pyrophosphate standard solution into 450 µL of ddH_2O or 50 mM Hepes buffer to get 100 µM Pyrophosphate standard solution (PS7). Take 100 µM Pyrophosphate standard solution and perform 1:3 serial dilutions in ddH_2O or 50 mM Hepes buffer to get serially diluted Pyrophosphate standards (PS6 - PS1).

PREPARATION OF WORKING SOLUTION

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

Note Due to the high sensitivity of this assay to PPi, it is important to use PPi-free labware and reagents. DTT ≥ 1 mM will increase the background, $MgCl_2$ ≥ 2mM will 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 (PS1 - PS7, 0.3 to 100 µM), 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 Dilutions (0.3 to 100 µM)
BL	50 µL	Assay Buffer
TS	50 µL	test sample

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.

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

3. Incubate at room temperature for 10 to 30 minutes.

4. Monitor the fluorescence increase with a fluorescence plate reader at Ex/Em = 316/456 nm (Cutoff = 420 nm).

EXAMPLE DATA ANALYSIS AND FIGURES

The reading (Control %) 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, ATP, Pi samples. We recommend using the Online Four Parameter Logistics Calculator which can be found at:

https://www.aatbio.com/tools/four-parameter-logistic-4pl-curve-regression-online-calculator

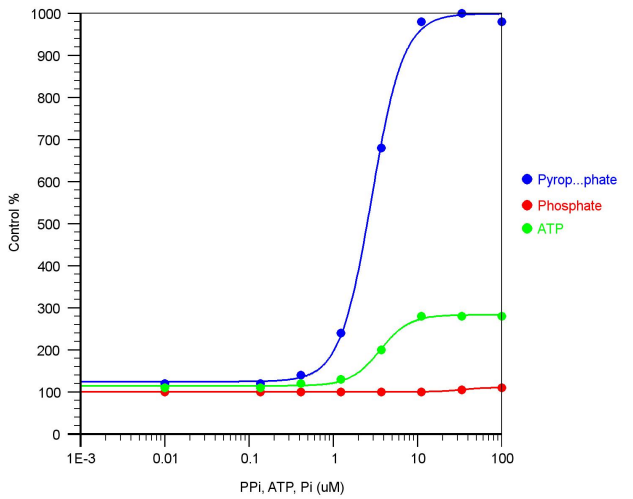

Figure 1. Pyrophosphate, ATP and phosphate dose responses were measured with PhosphoWorks™ Fluoremetric Pyrophosphate Assay Kit in a solid black 96-well plate using a fluorescence microplate reader.

DISCLAIMER