PhosphoWorks™ Fluorimetric Pyrophosphate Assay Kit
Blue Fluorescence

Ordering Information:
Product Number: #21611 (200 assays)

Instrument Platform:
Fluorescence microplate readers

Storage Conditions:
Keep in 4 °C and avoid light

Introduction

Pyrophosphate (PPi) are 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. The assay can be performed in a convenient 96-well or 384-well microtiter-plate format and easily adapted to automation with no separation steps required.

Kit Features and Benefits

Universal:	Can be used for monitoring any biological processes that generate pyrophosphate.
Continuous:	Easily adapted to automation with no mixing or separation.
Convenient:	Formulated to have minimal hands-on time.
Non-Radioactive:	No special requirements for waste treatment.

Kit Components

Component	Amount
Component A: Assay buffer	1 bottle (25 mL)
Component B: PPi senor	1 vial (lyophilized powder)
Component C: Pyrophasphate standard	1 mL (50 mM)
Component D: DMSO	1 vial (200 µL)

Assay Protocol (for one 96-well plate)

Brief Summary

Prepare test samples (50 µL) and pyrophosphate standards (50 µL)
→ Add Assay solution (50 µL)→ Incubate at room temperature for 10 to 30 min
→ Read Fluorescence at Ex/Em =368/415nm

1. Prepare Assay Reagents

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

1.2 Prepare 200X PPi sensor stock solution: Add 50 µL of DMSO (Component D) into the PPi sensor vial (Component B) to make 200X stock solution.
Note: 25 µL of the stock solution is enough for one 96-well plate. The unused PPi sensor stock solution should be divided as single use aliquots and stored at -20°C and avoid from light.

1.3 Prepare Assay Solution: Add 25 µL of 200X PPi substrate stock solution (from step 1.2) to 5 mL Assay Buffer (Component A), and mix them well to make the Assay Solution.
Note: Due to the high sensitivity of this assay for PPi, it is important to use PPi-free labware and reagents.

2. Prepare Pyrophosphate Standards and Test Samples

2.1 Prepare 1 mM pyrophosphate standard solution: Add 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 solution.

2.2 Take 50 µL of 1 mM pyrophosphate solution (from step 2.1) into 450 µL of assay buffer (Component A) to get 100 uM pyrophosphate solution, then take 200 µL of 100 uM pyrophosphate solution to perform 1:3 serial dilutions to give 30, 10, 3, 1, 0.3, 0.1 and 0 µM pyrophosphate solutions.

2.3 Add pyrophosphate-containing test samples and pyrophosphate standards into a 96-well solid black microplate as described according to Tables 1 and 2

Table 1. Layout of pyrophosphate standard and test samples in a clear 96-well microplate:

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

Note: PS=Pyrophosphate standard, BL=Blank control, TS=test sample.

Table 2. Reagent composition for each well:

Pyrophosphate Standard	**Blank Control**	**Test Sample**
Serial dilution* (50 µL)	Assay buffer (50 µL)	50 µL

*Note: *Add the serially diluted pyrophosphate from 0.3 µM to 100 µM into wells from PS1 to PS7.*

3. Run Pyrophosphate Assay

3.1 Add 50 µL/well of Assay solution (from Step 1.3) to the wells of pyrophosphate standards, blank control, and test samples. Mix the reagents completely.

Note: For a 384-well plate, add 25 µL sample and 25 µL Assay solution per well.

3.2 Incubate at room temperature for 10 to 30 min.

3.3 Monitor the fluorescence increase with 368 nm excitation and 415 nm emission with a fluorescence plate reader.

4. Data Analysis

The fluorescence in blank wells (with the assay buffer only) is used as a control, and is subtracted from the values for those wells with the pyrophosphate reactions. The typical data are shown in Figure 1 (pyrophosphate standard curve).

Note: The fluorescence background increases with time, thus it is important to subtract the fluorescence intensity value of the blank wells for each data point.

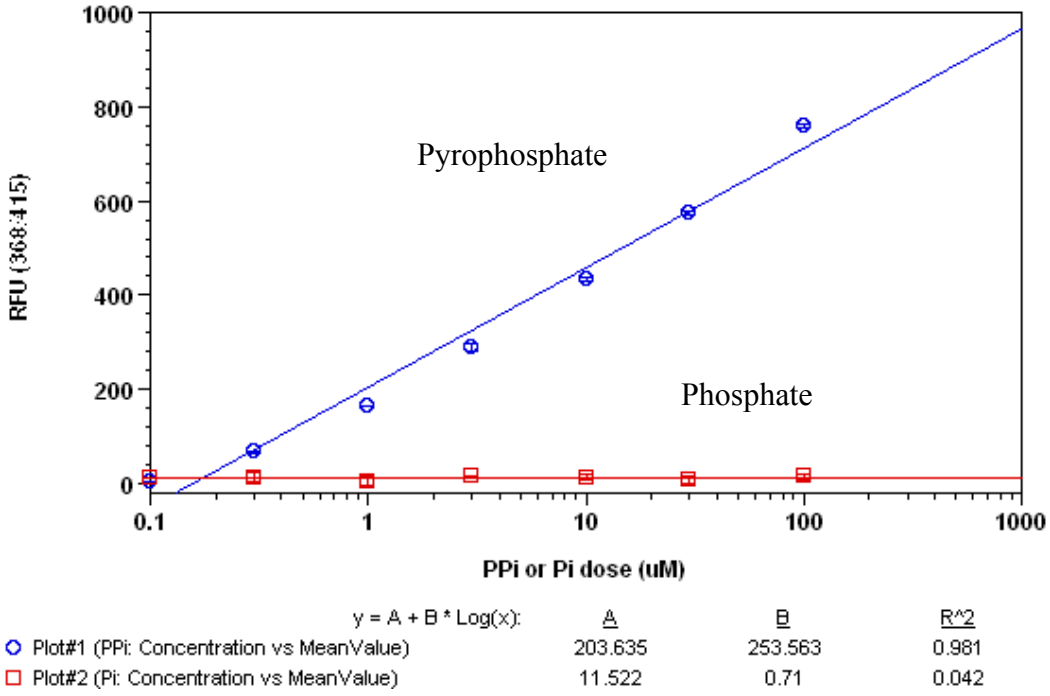

Figure1. Pyrophosphate and phosphate dose response on 96-well black plate was measured with the PhosphoWorks™ Pyrophosphate Assay Kit using a Gemini fluorescence microplate reader (Molecular Devices). As low as 0.3 µM (30 picmoles) pyrophosphate can be detected with 10 min incubation time.

Related Products

21665	PhosphoWorks™ Colorimetric Phosphate Assay Kit *Blue Color*	1 kit
21664	PhosphoWorks™ Colorimetric Phosphate Assay Kit *Yellow Color*	1 kit
21655	PhosphoWorks™ Fluorimetric ADP Assay Kit *Red Fluorescence*	1 kit
21658	PhosphoWorks™ Fluorimetric Phosphate Assay Kit *Blue Fluorescence*	1 kit
21660	PhosphoWorks™ Fluorimetric Phosphate Assay Kit *Red Fluorescence*	1 kit
21611	PhosphoWorks™ Fluorimetric Pyrophosphate Assay Kit *Blue Fluorescence*	1 kt
21612	PhosphoWorks™ Fluorimetric Pyrophosphate Assay Kit *Green Fluorescence*	1 kt
21610	PhosphoWorks™ Luminometric ATP Assay Kit *Bright Glow*	1 kit
21609	PhosphoWorks™ Luminometric ATP Assay Kit *Steady Glow*	1 kit

References

1. Zhou M, Diwu Z, Panchuk-Voloshina N and Haugland RP. (1997) A Stable Nonfluorescent Derivative of Resorufin for the Fluorometric Determination of Trace Hydrogen Peroxide: Applications in Detecting the Activity of Phagocyte NADPH Oxidase and Other Oxidases Anal Biochem 253, 162-168.
2. Mohanty, JG, Jaffe JS, Schulman E S,and RaibleDG. (1997) A highly sensitive fluorescent micro-assay of H_2O_2 release from activated human leukocytes using a dihydroxyphenoxazine derivative. *J. Immunol. Methods* 202: 133-141.

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