ab179836

Pyrophosphate Assay Kit II (Fluorometric)

Instructions for Use

For monitoring Pyrophosphate activity in cell culture and tissue extracts.

This product is for research use only and is not intended for diagnostic use.

Version: v2a Last Updated: 30 November 2023

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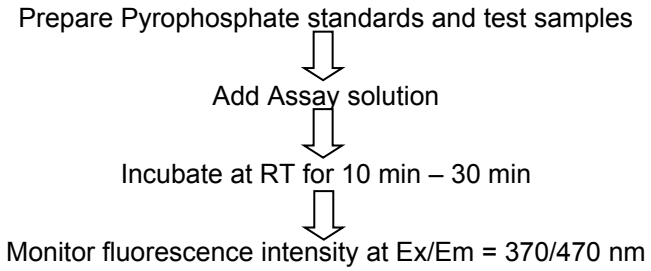
1. Overview

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. Pyrophosphate Assay Kit II (Fluorometric) (ab179836) provides the most robust spectrophotometric method for the measurement of pyrophosphate. It 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 enzyme-coupling pyrophosphate methods, which require at least two enzymes for their pyrophosphate detections. Due to its direct measurement of pyrophosphate, this kit is ideal for screening inhibition or activities of enzymes that consume or generate pyrophosphate with enhanced selectivity to pyrophosphate. The assay is an optimized mix-and-read assay and can be performed in a convenient 96-well or 384-well microtiter-plate format. The kit provides all the essential components for assaying pyrophosphate.

Abcam's Pyrophosphate Assay Kit II (Fluorometric) (ab179836) provides a simple, sensitive and rapid fluorescence-based method for detecting Pyrophosphate in cell culture and tissue extracts. The fluorescence signal can be read with a fluorescence microplate reader at Ex/Em = 370/470 nm.

2. Protocol Summary



3. Kit Components

Item	Quantity	Storage upon arrival	Storage after use/ reconstitution
Assay Buffer (25 mL)	1 bottle	-20°C	-20°C
PPi Sensor (lyophilized)	1 vial	-20°C	-20°C
Pyrophasphate Standard (50 mM)	1 mL	-20°C	-20°C
DMSO (100 µL)	1 vial	-20°C	-20°C

4. Storage and Stability

Upon arrival, store the kit at-20°C and protected from light. Please read the entire protocol before performing the assay. Avoid repeated freeze/thaw cycles.

Warm all buffers to room temperature before use. Briefly centrifuge all small vials prior to opening.

5. Materials Required, Not Supplied

- 96 or 384-well black plate with clear flat bottoms
- Multi-well spectrophotometer (ELISA reader)
- Distilled water (ddH_2O)or MilliQ
- Lysis Buffer

6. Assay Protocol

1. Reagent Preparation

a) PPi Sensor Stock Solution (200X):

Make a 200X PPi Sensor Stock Solution by adding 50 µL of DMSO into the vial of PPi Sensor to make 200X PPi Sensor stock solution. Pipette up and down to dissolve completely.

Note: 25 µL of the PPi Sensor Stock Solution is enough for one 96-well plate. The unused PPi Sensor Stock Solution should be divided into single-use aliquots. Store at -20°C and protect from light.

b) Assay Solution:

Add 25 µL 200X PPi Sensor Stock Solution (from step 1a) to 5 mL of Assay Buffer and mix well.*Note: Due to the high sensitivity of this assay to PPi, it is important to use PPi-free labware and reagents.*

Note: Samples such as plasma and serum might need a deproteinization step with spin columns

2. Pyophosphate Standards and Test Sample Preparation

a) Add 10 µL of 50 mM Pyrophosphate Standard into 490 µL of Assay Buffer, or buffer of your choice (preferably 50 mM Hepes buffer, pH 7) to make 1 mM pyrophosphate standard solution.

b) Take 200 µL of 1mM pyrophosphate standard solution to perform 1:3 serial dilutions to get 300, 100, 30, 10, 3, 1 and 0 µM serially diluted pyrophosphate standards with Assay Buffer.

c) Add serially diluted pyrophosphate standards and/or pyrophosphate-containing test samples into a solid black 96-well microplate, as described in the table below.

Layout of pyrophosphate standards and test samples in a solid black 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.

3. Run Pyrophosphate Assay

a) Add 50 µL/well of Assay Solution to the wells of pyrophosphate standards, blank control, and test samples into a solid black 96-well microplate as shown in the table below.

Note: For a 384-well plate, add 25 µL of Standard/sample and 25 µL of assay mixture into each well.

Reagent composition for each well

Pyrophosphate Standards	**Blank Control**	**Test Sample**
Serial Dilutions*: 50 µL	Assay Buffer: 50 µL	50 µL

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

b) Incubate the reaction at room temperature for 10 – 20 minutes, protected from light.

c) Measure the fluorescence increase with a fluorescence microplate reader at Ex/Em = 370/470 nm

7. 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. A pyrophosphate standard curve is shown in Figure 1. *The fluorescence background may increase with time, thus it is important to subtract the fluorescence intensity value of the blank wells for each data point.*

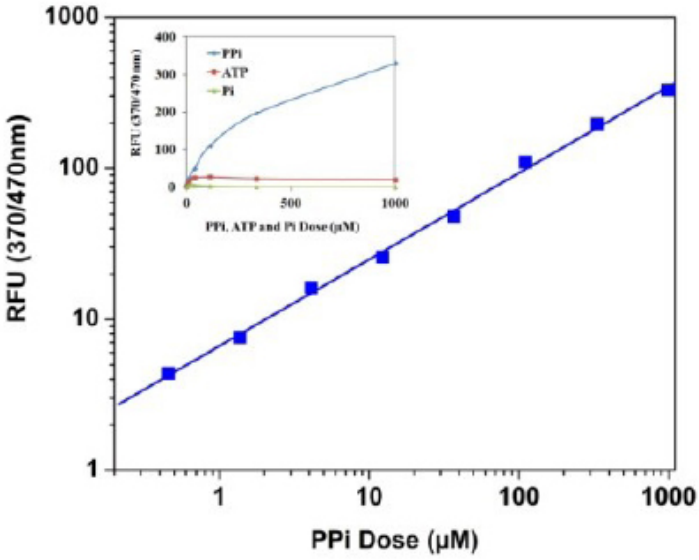


Figure 1. Pyrophosphate dose response was measured with Pyrophosphate Assay Kit II (Fluorometric) (ab179836) in a solid black 96-well using fluorescence microplate reader. As low as 1 µM (100 picomoles/well) pyrophosphate can be detected with 10 minutes incubation.

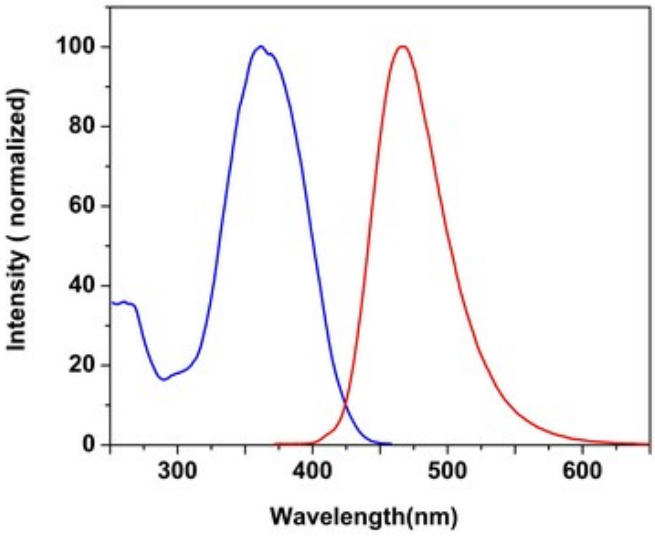


Figure 2. Excitation and emission spectra for Pyrophosphate Assay Kit II (Fluorometric) (ab179836).

8. Troubleshooting

Problem	Reason	Solution
Assay not working	Assay buffer at wrong temperature	Assay buffer must not be chilled - needs to be at RT
	Protocol step missed	Re-read and follow the protocol exactly
	Plate read at incorrect wavelength	Ensure you are using appropriate reader and filter settings (refer to datasheet)
	Unsuitable microtiter plate for assay	Fluorescence: Black plates (clear bottoms); Luminescence: White plates; Colorimetry: Clear plates. If critical, datasheet will indicate whether to use flat- or U-shaped wells
Unexpected results	Measured at wrong wavelength	Use appropriate reader and filter settings described in datasheet
	Samples contain impeding substances	Troubleshoot and also consider deproteinizing samples
	Unsuitable sample type	Use recommended samples types as listed on the datasheet
	Sample readings are outside linear range	Concentrate/ dilute samples to be in linear range

Problem	Reason	Solution
Samples with inconsistent readings	Unsuitable sample type	Refer to datasheet for details about incompatible samples
	Samples prepared in the wrong buffer	Use the assay buffer provided (or refer to datasheet for instructions)
	Samples not deproteinized (if indicated on datasheet)	Use the ***10kDa spin column (ab93349)***
	Cell/ tissue samples not sufficiently homogenized	Increase sonication time/ number of strokes with the Dounce homogenizer
	Too many freeze-thaw cycles	Aliquot samples to reduce the number of freeze-thaw cycles
	Samples contain impeding substances	Troubleshoot and also consider deproteinizing samples
	Samples are too old or incorrectly stored	Use freshly made samples and store at recommended temperature until use
Lower/ Higher readings in samples and standards	Not fully thawed kit components	Wait for components to thaw completely and gently mix prior use
	Out-of-date kit or incorrectly stored reagents	Always check expiry date and store kit components as recommended on the datasheet
	Reagents sitting for extended periods on ice	Try to prepare a fresh reaction mix prior to each use
	Incorrect incubation time/ temperature	Refer to datasheet for recommended incubation time and/ or temperature

	Incorrect amounts used	Check pipette is calibrated correctly (always use smallest volume pipette that can pipette entire volume)
Problem	**Reason**	**Solution**
Standard curve is not linear	Not fully thawed kit components	Wait for components to thaw completely and gently mix prior use
	Pipetting errors when setting up the standard curve	Try not to pipette too small volumes
	Incorrect pipetting when preparing the reaction mix	Always prepare a master mix
	Air bubbles in wells	Air bubbles will interfere with readings; try to avoid producing air bubbles and always remove bubbles prior to reading plates
	Concentration of standard stock incorrect	Recheck datasheet for recommended concentrations of standard stocks
	Errors in standard curve calculations	Refer to datasheet and re-check the calculations
	Use of other reagents than those provided with the kit	Use fresh components from the same kit

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