ab112155

Pyrophosphate Assay Kit (Fluorometric)

Instructions for Use

For the measurement of Pyrophosphate using our proprietary fluorescence probe for screening inhibition or enzyme activity

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

Version 7d Last updated 30 November 2023

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1. 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.

ab112155 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.

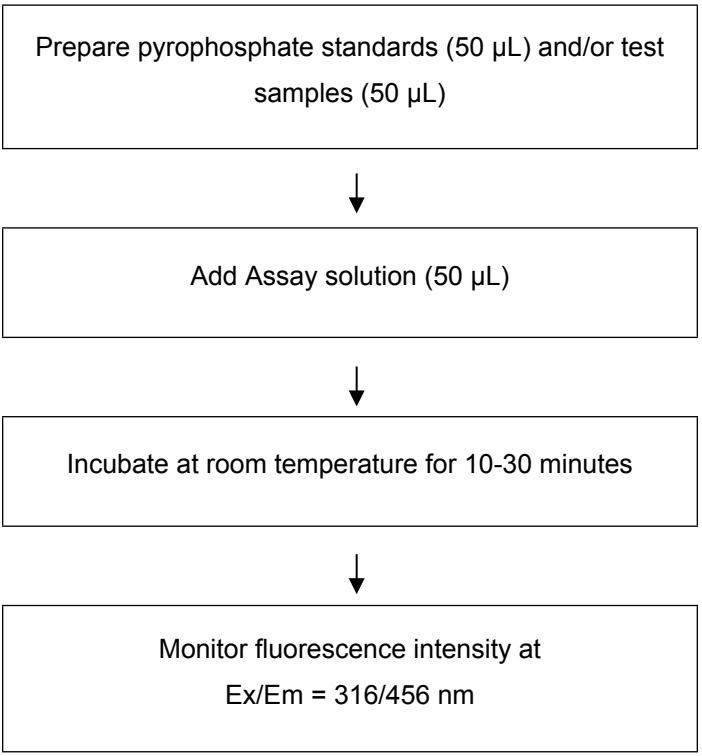
ab112155 is an optimized mix-and-read assay and can be performed in a convenient 96-well or 384-well microtiter-plate format. The kit provides all the essential components for assaying pyrophosphate.

Kit Key Features

- **Universal**: Can be used to monitor any biological processes that generate pyrophosphate.
- **Continuous:** Easily adapted to automation without mixing or separation.
- **Convenient**: Formulated to have minimal hands-on time.
- **Non-Radioactive**: No special requirements for waste treatment

2. Protocol Summary

Summary for One 96-well Plate



Note: Thaw all the kit components to room temperature before starting the experiment.

3. Kit Contents

Components	Amount
Component A: Assay Buffer	1 x 25 mL
Component B: PPi Sensor (Lyophilized)	1 vial
Component C: 50 mM Pyrophosphate Standard	1 mL
Component D: DMSO	1 x 200 µL

4. Storage and Handling

Keep at -20°C. Avoid exposure to light.

5. Assay Protocol

Note: This protocol is for one 96 - well plate.

A. Preparation of Assay Solutions

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

2. Prepare 200X PPi Sensor Stock Solution: 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. The unused PPi Sensor Stock Solution should be divided into single-use aliquots. Store at -20 °C and protect from light.

3. Prepare Assay Solution: Add 25 µL of 200X PPi Sensor Stock Solution (from Step A.2) 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: Samples such as plasma and serum might need a deproteinization step with spin columns

B. Preparation of Pyrophosphate Standards and Test Samples

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 standard solution.

2. Add 50 µL of 1 mM pyrophosphate standard solution (from Step 2.1) into 450 µL of Assay Buffer (Component A) to get 100 µM pyrophosphate standard solution, and then take 200 µL of 100 µM pyrophosphate standard solution to perform 1:3 serial dilutions to get 33.3, 11.1, 3.7, 1.2, 0.4, 0.13 and 0 µM serially diluted pyrophosphate standards.

3. Add serially diluted pyrophosphate standards and/or pyrophosphate-containing test samples into a solid black 96-well microplate as described in Tables 1 and 2.

4. Urine, serum and plasma samples should be diluted in a range of 1/250 -1/5000.

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

Table 1 Layout of pyrophosphate standards and test samples in a solid black 96-well microplate.

Note: PS = Pyrophosphate Standard, BL = Blank Control, TS = Test Sample

Pyrophosphate Standard	**Blank Control**	**Test Sample**
Serial dilutions*: 50 µL	Assay Buffer: 50 µL	50 µL

Table 2. Reagent composition for each well.

**Note: Add serially diluted pyrophosphate standards from 0.13 µM to 100 µM into wells from PS1 to PS7.*

C. Run Pyrophosphate Assay:

1. Add 50 µL/well of Assay Solution (from Step A.3) to the wells of pyrophosphate standards, blank control, and test samples. Mix the reagents thoroughly.

   Note: For a 384-well plate, add 25 µL of sample and 25 µL of Assay Solution into each well.

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

3. Measure fluorescence in a microplate reader at Ex/Em 316/456 nm.

6. 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 pyrophosphate reactions. A pyrophosphate standard curve is shown in Figure 1.

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.

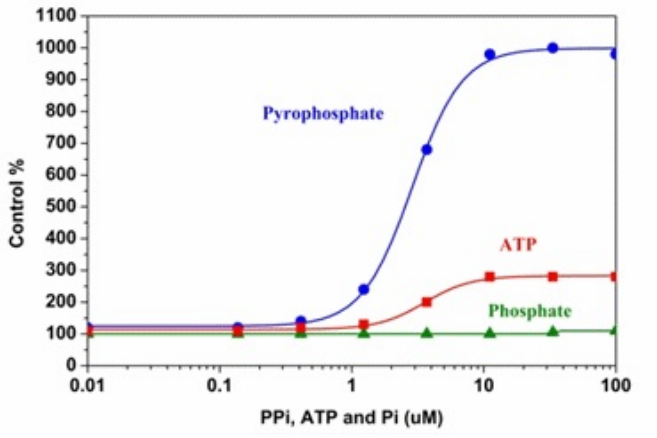


Figure 1. Pyrophosphate, ATP and phosphate dose responses were measured with ab112155 in a solid black 96-well plate using a fluorescence microplate reader. As low as 1 µM (100 picomoles/well) pyrophosphate can be detected with 10 minutes incubation.

7. 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)*** or ***Deproteinizing sample preparation kit (ab93299)***
	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)

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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