CREATIVE BIOMART®
Assay Kit

Pyrophosphate Fluorometric Assay Kit II

Product Information

Cat.No. Kit-0719

Product Overview

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).

Size

200 Tests

Kit Components

Component A: Assay Buffer, 1 bottle (25 mL), Freeze (<-15 °C)
Component B: PPi Sensor, 1 vial (lyophilized powder), Freeze (<-15 °C), Minimize light exposure
Component C: Pyrophosphate Standard, 1 vial (1 mL, 50 mM), Freeze (<-15 °C), Minimize light exposure
Component D: DMSO, 1 vial (200 µL), Freeze (<-15 °C)

Preparation

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 ddH2O or 50 mM Hepes buffer (pH 7) to make 1 mM Pyrophosphate standard solution.

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PREPARATION OF STANDARD SOLUTION
Add 50 µL of 1 mM Pyrophosphate standard solution into 450 µL of ddH2O 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 ddH2O 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 PPifree labware and reagents. DTT ≥ 1 mM will increase the background, MgCl2 ≥ 2mM will decrease the response.

Assay Protocol

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

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.

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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.
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).

Analysis

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.

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