

PhosphoWorks™ Fluorimetric Pyrophosphate Assay Kit

Blue Fluorescence with Enhanced Selectivity

Ordering Information

Product Number: 21614 (200 tests)

Storage Conditions

Keep in freezer and protect from light

Instrument Platform

Fluorescence microplate readers

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. AAT Bioquest's PhosphoWorks™ Fluorimetric Pyrophosphate Assay Kits provide 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.

Kit Components

Components	Amount
Component A: Assay Buffer	1 bottle (25 mL)
Component B: PPi Sensor	1 vial (lyophilized powder)
Component C: Pyrophosphate Standard	1 mL (50 mM)
Component D: DMSO	1 vial (100 µL)

Assay Protocol (for one 96-well plate)

Brief Summary

Prepare pyrophosphate standards (50 µL) and/or test samples (50 µL) → Add Assay solution (50 µL) → Incubate at room temperature for 10 to 30 minutes → Monitor fluorescence intensity at Ex/Em = 370/470 nm

1. Prepare assay solution:

- 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 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.
- 1.3 **Prepare Assay Solution:** Add 25 µL of 200X PPi Sensor Stock Solution (from Step 1.2) to 5 mL of Assay Buffer (Component A), and mix them well.
Note1: Due to the high sensitivity of this assay to PPi, it is important to use PPi-free labware and reagents.
Note2: DTT (1 µM) will increase the background, MgCl₂ ≥ 2mM decrease the response.

2. Prepare serially diluted 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 standard solution.
- 2.2 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 (Component A).
- 2.3 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.

