Selective Detection of Pyrophosphate Using a Fluorogenic Pyrophosphate Sensor

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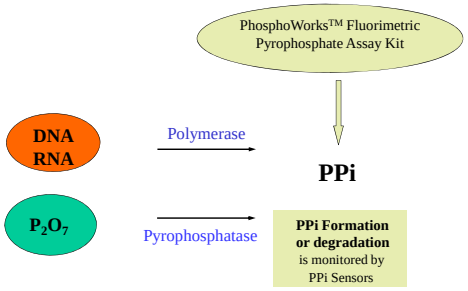
Introduction

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. Many pathological disorders such as familial chondrocalcinosis is associated with abnormal regulation of the transport of inorganic pyrophosphate. 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. The assay is much easier and more robust than the enzyme-coupling pyrophosphate methods that require at least two enzymes for their pyrophosphate detections. 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.

PhosphoWorks™ Pyrophosphatase Assay Principle



Enzymes that utilize PPi as a substrate such as phosphotransferases and pyrophosphatases or generate PPi such as cyclases, ligases, hydrolases, and DNA polymerase. The activities of these enzymes are determined by the monitoring of PP_i formation by PP_i sensors at Ex/Em=368nm/415nm.

Material and Methods

1. All Standard dilutions and pyrophasphate reactions were performed at room temperature with PhosphoWorks™ Fluorimetric Pyrophosphate Assay Kit.
2. Standard curves and pyrophasphate reactions were performed in 50 µl volumes in costar 96-solid black well plates for 30 min.
3. Pyrophosphate was measured as described in PhosphoWorks™ Fluorimetric Pyrophosphate Assay Kit by using a Gemini microplate reader (Molecular Devices) at EX 368/Em 415.

Prepare test samples and PPi standards (50 µL)

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Add assay solution (50 µL)

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Incubate at room temperature for 10 to 30 min

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Read fluorescence at Ex 368 nm/Em 415 nm

Results

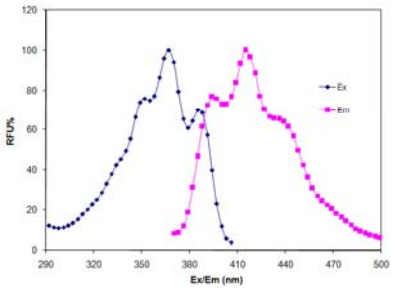

Figure1. The excitation and emission spectrum of pyrophosphate sensor

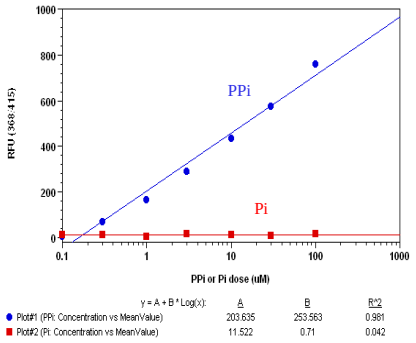

Figure 2. Pyrophosphate and phosphate dose response on 96-well black plate was measured with the PhosphoWorks™ Pyrophosphate Assay Kit using a Gemini fluorescence microplate reader (Molecular Devices). As low as 0.3 µM (30 picmoles) pyrophosphate can be detected in10 min incubation time.

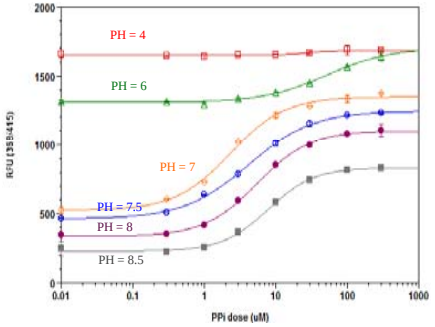

Figure 3. The pH effect of pyrophosphate sensor on pyrophosphate dose response.

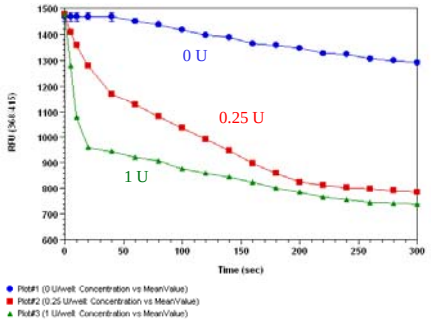

Figure 4. Inorganic pyrophosphatase detection with the PhosphoWorks™ Fluorimetric Pyrohosphate Assay Kit. The inorganic pyrophosphatase at 0, 0.25, and 1 units/well was incubated in the presence of 200 µM sodium pyrophosphate. The hydolysis of pyrophosphate generates the predicted fluorescence response was monitored as a function of time by using the PhosphoWorks™ Fluorimetric Phosphate Assay Kit.

Conclusions

1. **PhosphoWorks™ Fluorimetric Pyrophosphate Assay is a highly specific, sensitive, robust assay for measuring pyrophosphate.**
2. **As low as 0.3 µM pyrophosphate can be detected.**
3. **The optimal pH for pyrophosphate detection is 7 to 8.**
4. **The assay can be performed in a convenient 96-well or 384-well microtiter-plate format and easily adapted to automation with no separation required.**

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