Pyrophosphate Assay Kit (Fluorometric) ab112155

Overview

Product name: Pyrophosphate Assay Kit (Fluorometric)

Detection method: Fluorescent

Sample type: Urine, Serum, Plasma, Cell culture extracts, Adherent cells, Suspension cells, Tissue Lysate

Assay type: Quantitative

Assay time: 0h 30m

Product overview: Pyrophosphate Assay Kit (Fluorometric) ab112155 provides the most robust spectrophotometric method for the measurement of pyrophosphate.

The pyrophosphate assay protocol uses a proprietary fluorogenic pyrophosphate sensor that has its fluorescence intensity proportionally dependent upon the concentration of pyrophosphate.

This pyrophosphate assay protocol 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.

The pyrophosphate assay is an optimized mix-and-read assay and can be performed in a convenient 96-well or 384-well microtiter-plate format.

Notes:

This product measures PPi formation at Ex/Em = 316/456 nm.

If your instrument has filters for Ex/Em = 370/470 nm, we recommend using Pyrophosphate Assay Kit II (Fluorometric) (ab179836).

Pyrophosphates (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.

Platform: Microplate reader

Storage instructions: Store at -20°C. Please refer to protocols.
Urinary total pyrophosphate (PPi) concentrations were determined using ab112155 Caballero D et al., PLoS One, 12(7). Fig 1a. doi: 10.1371/journal.pone.0180098 Reproduced under the Creative Commons license http://creativecommons.org/licenses/by/4.0/

Urine PPi was corrected for urine creatinine, which was measured by LC-MS/MS or by ELISA using appropriate controls to adjust for inter-assay variability.

<table>
<thead>
<tr>
<th>Components</th>
<th>2 x 96 tests</th>
</tr>
</thead>
<tbody>
<tr>
<td>Assay Buffer</td>
<td>1 x 25ml</td>
</tr>
<tr>
<td>DMSO</td>
<td>1 x 200µl</td>
</tr>
<tr>
<td>PPI Sensor</td>
<td>1 vial</td>
</tr>
<tr>
<td>Pyrophosphate Standard (50mM)</td>
<td>1 x 1ml</td>
</tr>
</tbody>
</table>

Pyrophosphate measured in cell lysates showing quantity (nmol) per $10^6$ cells

Functional Studies- Pyrophosphate Assay Kit (Fluorometric) (ab112155)
Pyrophosphate measured in tissue lysates showing quantity (nmol) per microgram protein.

Pyrophosphate measured in biofluids showing concentration (micromolar). Obtained values were recalculated from 1:1000 sample dilutions.

Example PPI standard curve. Data provided for demonstration purposes only.
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.

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