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Product Information

High-Sensitivity Pyrophosphate Assay Kit

Catalog Number **MAK169**
Storage Temperature –20 °C

TECHNICAL BULLETIN

Product Description

Pyrophosphate (PP_i) is produced by a number of biochemical reactions such as ATP hydrolysis, DNA and RNA polymerizations, cyclic AMP formation, and the formation of fatty acid-coenzyme A esters.

The Pyrophosphate Assay Kit provides a simple and direct procedure for measuring pyrophosphate in a variety of samples. The pyrophosphate concentration of a sample is determined by the use of a unique fluorogenic pyrophosphate sensor in which the presence of pyrophosphate results in the production of a fluorescent product (λ_{ex} =370/λ_{em} = 470 nm) proportional to the pyrophosphate present. This assay is simpler and more robust than traditional enzyme-based methods and is ideal for screening enzyme activity or enzyme inhibitors.

Components

The kit is sufficient for 200 assays in 96 well plates.

Assay Buffer Catalog Number MAK169A	25 mL
PP_i Sensor Catalog Number MAK169B	1 vl
Pyrophosphate Standard, 50 mM Catalog Number MAK169C	1 mL
DMSO Catalog Number MAK169D	0.1 mL

Reagents and Equipment Required but Not Provided.

- 96 well flat-bottom plate – It is recommended to use black plates with clear bottoms for fluorometric assays.
- Fluorescence multiwell plate reader

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

The kit is shipped under ambient conditions and storage at –20 °C, protected from light, is recommended.

Preparation Instructions

Briefly centrifuge vials before opening. To maintain reagent integrity, avoid repeated freeze/thaw cycles.

Allow all reagents to come to room temperature before use.

Due to the high sensitivity of this assay, it is critical to use pyrophosphate-free labware and reagents.

PPi Sensor – Reconstitute with 50 µL of DMSO to make a 200× PPi Sensor stock solution. Mix well by pipetting (do not vortex), then aliquot and store, protected from light, at –20 °C.

Procedure

All samples and standards should be run in duplicate.

Pyrophosphate Standards

Add 10 µL of the 50 mM pyrophosphate standard solution to 490 µl of Assay Buffer to prepare a 1 mM pyrophosphate standard solution. Take 200 µL of the 1 mM pyrophosphate standard solution and prepare serial dilutions with Assay Buffer generating 300, 100, 30, 10, 3, 1, and 0 (blank) µM standards. Add 50 µL of each serially diluted standard into appropriate wells of a 96 well plate.

Sample Preparation

Add up to 50 µL of sample to wells. Bring samples to a final volume of 50 µL with Assay Buffer.

Note: For unknown samples, it is suggested to test several sample dilutions to ensure the readings are within the linear range of the standard curve.

Assay Reaction

1. Set up the Master Reaction Mix according to the scheme in Table 1. 50 µL of the Master Reaction Mix is required for each reaction (well).

Table 1.
Master Reaction Mix

Reagent	**Volume**
200× PP_i Sensor stock solution	25 µL
Assay Buffer	5 mL

Note: The Master Reaction Mix is enough for one plate and can be scaled if necessary.

2. Add 50 µL of the Master Reaction Mix to each of the sample, blank, and standard wells. Mix well using a horizontal shaker or by pipetting, and incubate the reaction for 10–30 minutes at room temperature. Cover the plate during the incubation.

3. Measure the fluorescence intensity (λ_{ex} =370/ λ_{em} = 470 nm.

Note: This assay can be adapted for use with 384 well plates. When working with 384 well plates, add 25 µL of standard, sample, and Master Reaction Mix to each well at the respective steps.

Results

Calculations

The background blank for the assay is the value obtained for the 0 (blank) pyrophosphate standard. Correct for the background by subtracting the blank value from all readings. Background values can be significant and must be subtracted from all readings.

Use the values obtained from the standards to plot a standard curve. The concentration of pyrophosphate present in the samples may be determined from the standard curve.

Note: A new standard curve must be set up each time the assay is run.

Troubleshooting Guide

Problem	Possible Cause	Suggested Solution
Assay not working	Cold Reagents	Assay Buffer must be at room temperature
	Omission of step in procedure	Refer and follow Technical Bulletin precisely
	Plate reader at incorrect wavelength	Check filter settings of instrument
	Type of 96 well plate used	For fluorometric assays, use black plates with clear bottoms
Samples with erratic readings	Samples prepared in an incompatible buffer	Use the Assay Buffer provided with the kit. Alternatively, a 50 mM HEPES buffer, pH 7, can be used.
	Presence of interfering substance in the sample	If possible, dilute sample further
	Use of old or inappropriately stored samples	Use fresh samples and store correctly until use
Lower/higher readings in samples and standards	Improperly thawed components	Thaw all components completely and mix gently before use
	Use of expired kit or improperly stored reagents	Check the expiration date and store the components appropriately
	Allowing the reagents to sit for extended times on ice	Prepare fresh Master Reaction Mix before each use
	Incorrect incubation times or temperatures	Refer to Technical Bulletin and verify correct incubation times and temperatures
	Incorrect volumes used	Use calibrated pipettes and aliquot correctly
Non-linear standard curve	Use of partially thawed components	Thaw and resuspend all components before preparing the reaction mix
	Pipetting errors in preparation of standards	Avoid pipetting small volumes
	Air bubbles formed in well	Pipette gently against the wall of the plate well
	Standard stock is at incorrect concentration	Refer to the standard dilution instructions in the Technical Bulletin
	Calculation errors	Recheck calculations after referring to Technical Bulletin
	Substituting reagents from older kits/lots	Use fresh components from the same kit
	Samples measured at incorrect wavelength	Check the equipment and filter settings
Unanticipated results	Samples contain interfering substances	If possible, dilute sample further
	Sample readings above/below the linear range	Concentrate or dilute samples so readings are in the linear range

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