Pyrophosphatase Inhibitor Assay Kit (BA0223)

Product Datasheet | SKU BA0223

Description

Inorganic pyrophosphatase (EC 3.6.1.1) catalyses the hydrolysis of phosphoester bonds of inorganic pyrophosphate, thereby releasing two orthophosphate molecules. Family I pyrophosphatases are essential enzymes found in all kingdoms of life and are responsible for maintaining the correct pyrophosphate equilibrium necessary to carry out nucleic acid and protein synthesis and to facilitate fatty acid beta oxidation. This inhibitor assay is based on a colorimetric pyrophosphatase assay and allows for screening of potential pyrophosphatase inhibitors.

Specifications

Detection Method	Colorimetric inhibitor screening; read at 620 nm
Detection Range	Not specified
Sample Type	Test compounds (inhibitors); pyrophosphatase enzyme
Species Reactivity	All
Assay Time	Approximately 70 minutes
Kit Size	100 Assays
Equipment Required	Microplate reader
Storage	Store pyrophosphate at -20 degrees C and store all other components at 4 degrees C upon receiving.
Shelf Life	6 months after receipt
Shipping	Room Temperature

Kit Components

Component	Quantity	Storage
Assay Buffer	8 mL	4 degrees C
Pyrophosphate	100 uL	-20 degrees C
Inhibitor	100 uL	4 degrees C
POMG Reagent A	2.5 mL	4 degrees C
POMG Reagent B	120 uL	4 degrees C

Ordering Information

Assay Genie Cat. No.	Pack Size
BA0223	100 Assays

Assay Procedure

Note: This is a sample protocol. Protocols are batch/lot specific - always follow the instructions supplied with your kit.

1. Dissolve test compounds in a solvent of choice (e.g. water, DMSO); it is prudent to first test the tolerance of any non-water solvent by the enzyme of choice, and yeast pyrophosphatase was found to tolerate up to 10% DMSO.
2. Prepare enzyme in an enzyme buffer such as 25 mM HEPES pH 7.2 with 5 mM MgCl2; the protocol is optimised for yeast pyrophosphatase, and enzyme should be diluted just prior to assay, running a serial enzyme dilution in water to determine the optimal concentration per well if using a different enzyme.
3. Avoid free phosphate (e.g. from detergent) in the enzyme buffer and test compounds as the assay is extremely sensitive; if the OD620nm for enzyme alone with the colour reagent is higher than 0.2, remove

free phosphate by at least 3 washes with a 10 kDa NMWL membrane filter. Avoid EDTA and metals such as Zn, Co, Mn and Ca, and note that high protein concentrations can interfere through precipitation.

4. Transfer 40 microlitres of Enzyme into separate wells of a clear flat-bottom 96-well plate, reserving at least one well for the No Inhibitor Control and one for the No Enzyme Blank, adding 40 microlitres of Enzyme sample and 40 microlitres of Assay Buffer to the Control and Blank wells respectively; add 10 microlitres of the test-compound solvent solution to the Control and Blank wells.
5. To the remaining enzyme-containing wells, add 10 microlitres of the test compounds, tap the plate to mix and incubate for 10 minutes at room temperature to allow the inhibitor to block enzyme activity.
6. Prepare enough Working Reagent for all wells by mixing 1 microlitre of pyrophosphate and 45 microlitres of Assay Buffer per well, transfer 40 microlitres of Working Reagent to all wells, briefly tap the plate to mix and incubate for 30 minutes at room temperature.
7. Prepare enough Colour Development Reagent for all wells by mixing 100 parts POMG Reagent A and 1 part POMG Reagent B, add 20 microlitres of Colour Development Reagent to all wells, tap the plate thoroughly to mix, incubate for 30 minutes at room temperature and read optical density at 620 nm.

Calculation

Relative enzyme activity for a test compound is calculated as: Activity (%) = [(OD_Test Cmpd - OD_Blank) / (OD_Control - OD_Blank)] x 100%, where OD_Test Cmpd, OD_Control and OD_Blank are the OD620nm values of the test compound, control and blank wells at 30 minutes.

Notes

- The assay is extremely sensitive to free phosphate; phosphate in the enzyme buffer and test compounds should be tested and/or avoided, and free phosphate can be removed using a 10 kDa NMWL membrane filter.
- Avoid EDTA and metals such as Zn, Co, Mn and Ca that may affect enzyme activity, and note that high protein concentrations can interfere with colour development through precipitation.
- Reagents are for research use only. Refer to the MSDS for detailed safety information.