QuantiChrom™ Pyrophosphatase Inhibitor Assay Kit (DPPI-100)

Quantitative Colorimetric Inhibitor Screening Assay for Pyrophosphatase

DESCRIPTION

INORGANIC PYROPHOSPHATASE(E.C.3.6.1.1) catalyzes the hydrolysis of phosphoester bonds of inorganic pyrophosphate [$P_2O_7^{4-}$], thereby releasing two orthophosphate molecules. Family I PPases 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 facilitate fatty acid beta oxidation.

BioAssay Systems' QuantiChrom™ Pyrophosphatase Inhibitor Assay is based on our pyrophosphatase assay kit (DPPT-100) and allows for screening of potential pyrophosphatase inhibitors.

KEY FEATURES

Safe. Non-radioactive assay.

Fast and convenient. The procedure involves incubation of enzyme and inhibitor, addition of a single working reagent and incubation for 30 min. Room temperature assay. No 37°C incubator is needed.

High-throughput. Homogenous "mix-incubate-measure" type assay. Can be readily automated on HTS liquid handling systems. Robust assay with a Z' factor of 0.59. Can be used in 96-well, 384-well, and potentially higher density screening assays.

APPLICATIONS

For high-throughput inhibitor screening and evaluation of Family I pyrophosphatase modulators.

KIT CONTENTS (100 TESTS IN 96-WELL PLATES)

Assay Buffer :	8 mL	**POMG Reagent A:**	2.5 mL
Pyrophosphate:	100 µL	**POMG Reagent B:**	120 µL
Inhibitor:	100 µL		

Storage Conditions. The kit is shipped at room temperature. Store pyrophosphate at -20°C, and store all other components at 4°C upon receiving. Shelf life: 6 months after receipt.

Precautions: Reagents are for research use only. Normal precautions for laboratory reagents should be exercised while using the reagents. Please refer to Material Safety Data Sheet for detailed information.

PROCEDURES

Test Compound Preparation. Dissolve test compounds in a solvent of choice (e.g. H_2O, DMSO). It is prudent to first test the tolerance of the solvent (if the solvent is not H_2O) by the enzyme of choice. In our testing, yeast pyrophosphatase (Sigma Aldrich Cat# I1643) was found to tolerate up to 10% DMSO.

Enzyme Preparation. Enzyme should be prepared in an enzyme buffer, e.g. 25 mM HEPES pH 7.2, 5 mM $MgCl_2$. The following protocol is optimized for yeast pyrophosphatase (Sigma Aldrich Cat# I1643). Enzyme should be diluted just prior to assay. If using a different enzyme, we recommend running a serial enzyme dilution in dH_2O to determine optimal enzyme concentration to use per well.

***Note**: 1. Phosphate in enzyme buffer (e.g. from detergent) and test compounds should be tested and/or avoided as the assay is extremely sensitive to free phosphate. If the OD620nm for enzyme alone with the color reagent is higher than 0.2, we recommend removing free phosphate by at least 3 washes with a 10 kDa NMWL membrane filter (e.g. Amicon® Ultra 0.5 mL Ultracel or similar). 2. Avoid EDTA and metals such as Zn, Co, Mn, and Ca that may affect the enzyme activity. 3. High protein concentrations can interfere with the Color Development through precipitation.*

Inhibitor Screening in 96-Well Plate

1. Transfer 40 µL of Enzyme into separate wells of a clear, flat-bottom 96-well plate. Reserve at least one well for No Inhibitor Control ("Control") and one for the No Enzyme Blank ("Blank"): Add 40 µL of Enzyme sample and 40 µL of Assay Buffer to the Control and Blank wells respectively.

   To the Control and Blank wells, add 10 µL of the solvent solution that the test compounds are dissolved in. For example, if the test compounds are dissolved in 0.5% DMSO, add 10 µL of this solution to these wells.
2. To the remainder of the wells containing enzyme, add 10 µL of the test compounds. Tap plate to mix (make sure enzyme and inhibitor are mixed) and incubate for 10 min at RT to allow the inhibitor to block enzyme activity.
3. Prepare enough Working Reagent (WR) for all wells by mixing 1 µL of pyrophosphate and 45 µL of Assay Buffer for each well. Transfer 40 µL of WR to all wells. Briefly tap plate to mix. Incubate for 30 min at room temperature.
4. Prepare enough Color Development Reagent for all wells by mixing 100 parts POMG Reagent A and 1 part POMG Reagent B. Add 20 µL Color Development Reagent to all wells. Tap plate thoroughly to mix.

   Incubate for 30 minutes at room temperature and read optical density OD620nm.

CALCULATION

Relative enzyme activity for a test compound is calculated as follows:

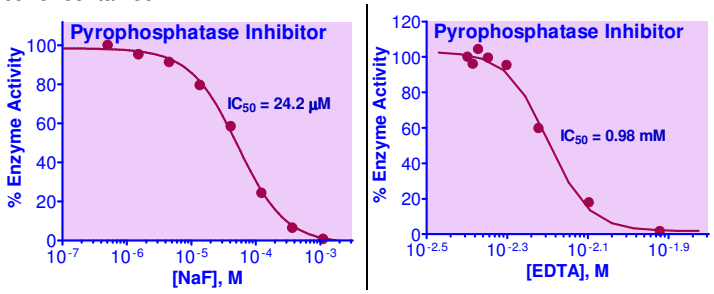
$$\text{Activity (\%)} = \frac{OD_{\text{Test Cmpd}} - OD_{\text{Blank}}}{OD_{\text{Control}} - OD_{\text{Blank}}} \times 100\%$$

Where $OD_{\text{Test Cmpd}}$, OD_{Control} and OD_{Blank} are the OD620nm values of the test compound, the control, and the blank wells at 30 min.

MATERIALS REQUIRED, BUT NOT PROVIDED

Pyrophosphatase, pipetting (multi-channel) devices. Clear-bottom 96-well plates (e.g. Corning Costar) and plate reader.

EXAMPLES. Pyrophosphatase from yeast (Sigma Aldrich cat# I1643) at 10 U/L was incubated with NaF and EDTA at various concentrations for 10 min at RT. Enzyme reaction and detection were carried out according to the standard procedure in a 96-well plate. Note: the assay buffer contained EDTA



LITERATURE

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3. Nakano, T., et al (1999) Purification and Characterization of Phytase from Bran of *Triticum aestivum* L. cv. Nourin #61. *Food Sci. Technol. Res.* 5(1): 18.

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