CREATIVE BIOMART®
Assay Kit

GTPase Kinetic Assay Kit

Product Information

Cat.No. Kit-0401

Product Overview

There are a multitude of enzymes that hydrolyze ATP or GTP to form ADP or GDP and inorganic phosphate (Pi). The Enzyme Linked Inorganic Phosphate Assay (ELIPA) from Cytoskeleton Inc. allows one to measure the phosphate released during hydrolysis on a real time basis. Thus a kinetic assay technique is produced for your enzyme"s activity. Particular purified enzymes that are applicable to this analysis are signaling phosphatases, apyrase, kinesin motors (see Fig. 1), metabolic enzymes, membrane transporters and alkaline phosphatase. Generally this assay is useful for enzymes with Kcat above 0.1, if your enzyme or the family of enzymes has a lower Kcat then the CytoPhosTM Phosphate Assay is useful. If your enzyme has a very low activity i.e. small G-proteins, the non-radioactive GAP assay or the radioactive alternative, EasyRad Phosphate Assay is ideal.The assay is an adaptation of a method originally described by Webb for the measurement of glycerol kinase plus D-glyceraldehyde ATPase activity and for actin activated myosin ATPase. The assay is based upon an absorbance shift (330-360 nm) that occurs when 2-amino-6-mercapto-7-methylpurine ribonucleoside (MESG) is catalytically converted to 2-amino-6-mercapto-7-methyl purine in the presence of inorganic phosphate (Pi). The reaction is catalyzed by purine nucleoside phosphorylase (PNP). One molecule of inorganic phosphate will yield one molecule of 2-amino-6-mercapto-7-methyl purine in an essentially irreversible reaction. Thus, the absorbance at 360 nm is directly proportional to the amount of Pi generated in the reaction.

Size

96 assays

Applications

1. Kinetic measurement of ATPase and GTPase activities2. Discovery and characterization of ATPase/GTPase inhibitors

Storage

The kit arrives at room temperature and can be stored desiccated at 4°C.

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Kit Components

1. Reaction Buffer: One bottle containing 30 ml of buffer. Composition, 15 mM PIPES pH 7.0, 5 mM MgCl2.2. ELIPA Reagent 1: One bottle, lyophilized. Contains 20 µmoles of 2-amino-6-mercapto-7-methylpurine riboside (MESG). 3. ELIPA Reagent 2: One tube. Contains 50 units of purine nucleoside phosphorylase (PNP). One unit of PNP will cause the phosphorolysis of 1 µmole of inosine to hypoxanthine and ribose 1-phosphate per minute at pH 7.4 at 25°C. 4. Phosphate Standard: One tube. Contains 1 ml of 0.5 mM phosphate standard (KH2PO4). 5. ELIPA Reagent 1 Resuspension Buffer: One bottle. Contains 20 ml of 3.0 mM acetic acid. 6. ATP Stock: One tube, lyophilized. When reconstituted; 1 ml of 100 mM ATP. 7. GTP Stock: Two tubes, lyophilized. When reconstituted; 100 µl of 100 mM GTP per tube.

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