CREATIVE BIOMART®
Assay Kit

Continuous ITP pyrophosphatase Assay Kit

Product Information

Cat

Kit-0876

Common Name

ITPA

Cat.No. Kit-0876

Product Overview

ITPase Assay Kit is the first non-radioactive and continuous kit designed to measure ITP pryophosphohydrolase content in samples. This enzymatic assay is based on a reaction involving Inosine Monophosphate Dehydrogenase (IMPDH).
The principle of the assay is based on the coupling of the following enzymatic reactions:
(1) In the presence of ITP, ITP pyrophosphohydrolase enzyme catalyzes the formation of IMP.
(2) In the presence of NAD, IMP is immediately oxidized by a highly active IMPDH in the presence of NAD with simultaneous formation of NADH2 directly monitored spectrophotometrically at 340 nm.
The assay is developed for measuring ITPase activity in vitro or in cell lysates.
For maximal accuracy, the assays with cell lysates are run with and without ITP in parallel. The absorbance rate observed in the absence of ITP is used as blank and is subtracted from the absorbance rate measured in its presence.

Description

ITP pyrophosphatase, or ITPase (EC 3.6.1.19), is an intracellular enzyme that catalyzes the pyrophosphohydrolysis of ITP/dITP and xanthosine triphosphate to prevent unusual nucleoside triphosphates from accumulating in (deoxy) nucleoside triphosphate (d)NTP pools and being integrated into RNA and DNA. This enzyme is encoded by the ITPA gene in mammals. Certain ITPA variants causing ITPase deficiency have been linked to adverse reactions to the immunosuppressive thiopurine drugs azathioprine and 6- mercaptopurine, and protection against ribavirin-induced anemia.

Storage

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Once dissolved, the reagents provided in the kit are not stable and should be stored on ice and used the day of preparation.

Size

24 analysis

Kit Components

The kit allows to perform 24 analyses in a time (8 samples in triplicate, 12 samples in duplicate).
A standard ITPase Assay Kit contains:
one tube "Cofactor 1";
one tube "Cofactor 2 ";
one tube "Enzymatic mix";
one tube "10X buffer" (pre-filled with 1 ml of 10X buffer);
one 15mL tube "Blank" orange cap;
one 15mL tube "Reaction mixture with ITP" blue cap (pre-filled with 5µmol ITP);
one transparent 96-well plate.

Materials Required but Not Supplied

1) Plate agitator
2) Plate reader fitted with a filter 340nm, Epoch; PerkinElmer.

Preparation

1. Transfer the content of the tube "10X buffer" into the 15mL tube "Blank" (orange cap) and add 9mL of deionized water. 10mL of 1X buffer is obtained.
2. Quantitatively transfer the content of 3 tubes with "Cofactor 1", "Cofactor 2", and "Enzymatic mix" to "Blank" tube.
To do so:
- pipet 1ml of buffer from "Blank" to each tube and mix them by inverting or pipeting up and down until the powder is dissolved.
- transfer the content of the tubes back into a vial "Blank" by pipeting.
- repeat to be sure that all reagents and enzymes of the small tubes and vial are recovered. Mix by gently inverting until complete dissolution. Avoid bubbles.

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3. Transfer 5ml of complete "Reaction mixture 1x" containing enzymes and cofactors to blue cap 15ml tube pre-filled with ITP.
You have prepared: 5ml of "Blank"; 5ml of "Reaction mixture with 1mM ITP".

Assay Protocol

1. Preparation of hemolysates. The pellet of PBS-washed erythrocytes from 100µL of blood was frozenthawed twice, resuspended in 500µL of ice-cold deionized water and used directly for ITPase quantification.
2. Add 5µL of hemolysates per well.
3. Add 200µL of "Blank" per well and 200µL of "Reaction mixture" containing 1mM ITP.
4. Program plate reader for kinetics absorbance reading (every 2min), 37°C.
Insert the plate into the reader pre-heated at 37°C, agitate for 1min and monitor the reaction at 340nm at 37°C for 1 hour with data collection every 2min.

Analysis

1. Calculate the absorbance rate per hour for reaction buffers with ITP (ARITP) and without (ARblank).
2. Calculate Mean ARITP and Mean ARblank.
3. Measure the concentration of hemoglobin [Hgb] in hemolysates using Drabkin's reagent and calculate final [Hgb] concentration used in assay.
4. ITPase activity is calculated by the following formula:
Activity = (Mean ARITP-Mean ARblank)/ (4.9x[Hgb])x 103 = (0.463-0.014)/(4.9 x0.97)x 103 = 94.5 nmol/hour/mg of Hgb.
where: MeanARITP=0.463; MeanARblank=0.014; [Hgb], final haemoglobin concentration used in assay=0.97mg/ml.
4.9 is the absorbance of 1mM NADH at 340nm in 200μL-round-bottom well of 96-well microplate.

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