CREATIVE BIOMART®
Assay Kit

PNP Assay Kit

Product Information

Cat

Kit-0877

Common Name

PNP

Cat.No.

Kit-0877

Product Overview

PNP Assay Kit provides an enzymatic tool allowing direct continuous spectrophotometric monitoring of PNP activity in a convenient 96-well plate format. In the assay, PNP activity is measured as a rate of production of hypoxanthine, which is directly oxidized by XDH enzyme with concomitant reduction of NAD+ to NADH measurable by absorbance at 340nm.
The assay is developed for measuring PNP activity in vitro or in cell lysates.
For maximal accuracy, the assays with cell lysates are run with and without inosine in parallel. The absorbance rate observed in the absence of inosine is used as blank and is subtracted from the absorbance rate measured in its presence.

Description

Purine nucleoside phosphorylase (PNP, EC 2.4.2.1) is involved in salvage pathway of the purine metabolism. In the presence of inorganic phosphate PNP catalyzes the cleavage of the glycosidic bond of ribo- or deoxyribonucleosides (inosine, guanosine or their deoxyanalogues) to generate the purine base and ribose- or deoxyribose-1-phosphate. The reaction is reversible for natural substrates. Purine nucleoside phosphorylase deficiency is a rare autosomal recessive metabolic disorder which results in accumulation of toxic metabolites in T-cell lymphocytes and severe immunodeficiency. In addition, purine nucleoside phosphorylase deficiency is associated with neurologic symptoms, including mental retardation and muscle spasticity, and increased risk of autoimmune disorders. Since most parasitic protozoan are obligate auxotrophs of purines and entirely depend therefore on their purine salvage pathways, PNP of apicomplexan parasites (P. falciparum and T. gondii) is

Tel: 1-631-559-9269 1-516-512-3133
Email:info@creative-biomart.com
Fax:1-631-938-8127
45-1 Ramsey Road, Shirley, NY 11967, USA

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currently explored as a potential target for drug development.

Storage

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 analysis in a time (8 samples in triplicate, 12 samples in duplicate).
A standard PNP Assay Kit contains:
one 2mL-tube "Cofactor 1" (DTT);
one 2mL-tube "Cofactor 2" (NAD);
one vial "XDH Enzyme";
one 15mL tube "Blank" (pre-filled with 10mL of "Reaction buffer");
one 15mL tube "Reaction mixture" (pre-filled with 50μL of 100mM inosine);
one tube of "Purified PNP" (human recombinant) to be used as a positive control;
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. Add 500μL of deionized water to the tube with "Cofactor 1" to prepare 0.1M DTT. Agitate until complete dissolution of the powder. Transfer dissolved "Cofactor 1" to the tube with "XDH enzyme". Agitate gently until complete dissolution of the powder
2. Quantitatively transfer the content of the tubes with "Cofactor 2" and solubilized "XDH enzyme" to a 15-ml tube "Blank".
To do so:
-pipet 1ml of buffer from "Blank" to each of 2 tubes and mix them by inverting or pipeting up and down until the powder dissolved.

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- transfer by pipeting the content of two tubes back into a vial "Blank".
- repeat to be sure that all reagent and enzymes of the small tubes and vial are recovered. mix by gently inverting until complete dissolution. Avoid bubbles.
3. Transfer 5mL of "Blank" solution to "Reaction mixture" tube pre-filled with inosine.
You have prepared: 5ml of "Blank"; 5ml of "Reaction mixture" containing 1mM inosine.
Preheat both mixtures at 37°C for 15 mintues.

Assay Protocol

1. Preparation of hemolysates. The pellet of PBS-washed erythrocytes from 100μL of blood was frozenthawed twice, resuspended in 2mL of ice-cold deionized water and used directly for PNP quantification.
2. Positive control. Add indicated volume of deionized water (1.8mL) to "Purified PNP" enzyme (human recombinant, 1.3Units, provided lyophilized) to obtain solution at 85 μmol/h/mL and mix gently until the powder is dissolved. Once dissolved, PNP should be used immediately. Add 4μL of resuspended PNP enzyme per well.
3. Add 4μL of hemolysates per well.
4. Add 200μL of "Blank" per well and 200μL of "Reaction mixture" containing 1mM inosine.
5. Program plate reader for kinetics absorbance reading (every 1 min), 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 20min with data collection every 1min.

Analysis

A. Calculation of activity of human recombinant PNP (positive control)
1. Calculate the absorbance rate per hour for reaction buffers with inosine (ARINO) and without (ARblanc).
2. Calculate Mean ARINO and Mean ARblank.
3. Calculate PNP activity as follows:
PNP Activity (in nmol/ml/hour) = (ARINO-ARblank)/4.9x103 = (4.555-0.116)/4.9x103 = 905.9 nmol/ml/h.
where: MeanARINO = 4.555; MeanARblank = 0.116.
4.9 is the absorbance of 1mM NADH at 340nm in 200μL-round-bottom well of 96-well microplate.
B. Calculation of PNP activity in hemolysates

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1. For first two hours, calculate the absorbance rate per hour for reaction buffers with inosine (ARINO) and without (ARblank). Calculate Mean ARINO and Mean ARblank.
2. Measure the concentration of hemoglobin [Hgb] in sonicated hemolysates using Drabkin's reagent and calculate final [Hgb] concentration used in assay.
3. PNP activity is calculated by the following formula:
Activity = (Mean ARINO-Mean ARblank)/(4.9x[Hgb])x103 = (1.187+0.021)/(4.9x0.24)x103 = 1027 nmol/hour/mg of Hgb.
where: MeanARINO=1.187; MeanARblank=-0.021; [Hgb], final haemoglobin concentration used in assay=0.24mg/ml.
4.9 is the absorbance of 1mM NADH at 340nm in 200μL-round-bottom well of 96-well microplate.

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