CREATIVE BIOMART®
Assay Kit

Continuous PRPP-S Assay Kit

Product Information

Cat.No. Kit-0871

Product Overview

PRPP-S Assay Kit is designed for continuous monitoring of PRPP synthesis. The assay is based on coupling of two recombinant enzymes : Hypoxanthine-guanine phosphoribosyltransferase (HGPRT) and Inosine Monophosphate Dehydrogenase (IMPDH).
The principle of the assay is based on the coupling of the following enzymatic reactions:
(1) In the presence of ATP and P-ribose, PRPP-Synthetase enzyme catalyzes the formation of PRPP.
(2) In the presence of Hypoxanthine (Hx), PRPP is converted to IMP by Hypoxanthine-guanine phosphoribosyltransferase (HGPRT).
(3) IMP is immediately oxidized by a highly active IMPDH in the presence of NAD with simultaneous formation of NADH2 directly monitored spectrophotometrically at 340nm.
The assay is developed for measuring PRPP-S activity in vitro or in cell lysates. For maximal accuracy, the assays with cell lysates are run with and without P-ribose 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.

Storage

The kit is shipped at room temperature since dry reagents and lyophilized enzymes are stable at room temperature (up to 2 weeks). However, for long time storage the kit should be frozen upon arrival and stored at -20°C.

Size

24 analysis

Kit Components

Cysteine (12mg, powder);
NAD (17mg, powder)
ATP (33mg, powder)
P-ribose (10mg, powder)
HPRT and IMPDH enzymes, lyophilized

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Reaction buffer (glass vial, 10mL) ;
one transparent 96-well plate
Once dissolved, the reagents can be stored at -20°C for three months.

Materials Required but Not Supplied

1) Plate agitator
2) Plate reader fitted with a filter 340nm

Assay Protocol

Preparation of 1ml "Reaction mixture"
IMPORTANT: Use only autoclaved Milli-Q water to inactivate ubiquitous phosphatases and to avoid dephosphorylation of P-ribose and PRPP present in reaction mixture
1. Shortly spin the tubes before opening to recover the powder at the bottom;
2. Thaw "Reaction buffer" (do not heat); equilibrate at room temperature ;
3. Add 200µL of deionized water to the tube with "HPRT and IMPDH enzymes", agitate (do not vortex to avoid foam) and spin shortly;
4. Add 100µL of deionized water to each of four tubes (Cysteine, NAD, ATP and P-ribose). Vortex until complete dissolution, spin shortly;
5. Put 0.85mL of reaction buffer in a clean 1.5mL tube, add 9µL of "Cysteine", 9µL of "NAD" and 9µL of "ATP" solutions. Do not add P-ribose solution
6. Close and agitate by inverting;
7. Add 18µL of "HPRT-IMPDH enzyme" solution; close and agitate by inverting, spin shortly;

Composition of reaction mixture: 100mM Tris-HCl, 100mM KCl, 12mM $MgCl_2$, BSA 1mg/ml, 2,5mM NAD, 7,5mM cysteine, 4mM ATP, IMPDH-HGPRT 30mU/ml each, pH8.5, start by P-ribose (3.5mM).

Reaction monitoring
1. Program plate reader for kinetics absorbance reading (every 1 min), 37°C.
2. Add desired amount of PRPP-S solution (1-10µL) per well to four wells, followed by addition of 200µL of "Reaction mixture"
3. Insert the plate into the reader pre-heated at 37°C, agitate for 1min and incubate for 15 min;

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4. To start the reaction, add 2μL of P-ribose solution to two wells (two other will be used as Blank), agitate and monitor the reaction at 340nm at 37°C for 30 min-1 hour with data collection every min.

Analysis

1. Calculate the absorbance rate per hour for reaction buffers with Ribose 5-phosphate (ARP5R) and without (ARblank).
2. Calculate Mean ARP5r and Mean ARblank
3. Calculate PRPP-S activity using following formula:

Activity= (Mean ARP5r-Mean ARblank) / (4.9x[Hgb]) x1000 = (0.229-0.014)/(4.9x0.62)x1000 =71 nmol/hour/mg of Hgb

where: Mean ARP5r=0.229;

Mean ARblank=0.014;

[Hgb], final haemoglobin concentration used in assay=0.62mg/ml.

4.9 is the absorbance of 1mM NADH at 340nm in 200μL-round-bottom well of 96-well microplate.

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