CREATIVE BIOMART®
Assay Kit

HPRT Assay Kit

Product Information

Cat

Kit-0874

Common Name

HPRT

Cat.No.

Kit-0874

Product Overview

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

Description

Hypoxanthine phosphoribosyltransferase is a purine salvage enzyme that catalyzes the reversible transfer of the 5-phosphoribosyl moiety from a-D-5-phosphoribosyl-1-pyrophosphate (PRPP) to a purine base (hypoxanthine or guanine) to form a nucleoside monophosphate (inosine monophosphate or guanosine monophosphate, respectively). In the presence of pyrophosphate, HPRT enzyme catalyzes also the hydrolysis of IMP and GMP, although this reverse reaction is much less favored than forward one.
Human HPRT enzyme does not hydrolyse XMP. HPRT1 gene is one of the best characterized in the human genome for two reasons: (i) HPRT1 gene is widely used as a somatic cell genetic marker in genotoxicity / mutagenicity studies; (ii) the defects within the human enzyme are associated with inherited gouty arthritis and Lesch–Nyhan syndrome and more than 300 disease-associated mutations in human HPRT1 gene leading to partial or complete deficiencies of the HPRT enzyme

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have been described. In view of the high variability of HPRT1 gene, a rapid biochemical assay would be useful both for basic science and clinical research.
In addition, since most parasitic protozoan are obligate auxotrophs of purines and entirely depend therefore on their purine salvage pathways, protozoan HPRT enzyme is an attractive target for the discovery of new anti-parasitic drugs. The enzymatic microplate assay enabling monitoring of HPRT activity may therefore accelerate the search of new anti-parasitic drugs.

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

This kit allows performing 24 analysis in a time (8 samples in triplicate, 12 samples in duplicate).
A standard HPRT Assay Kit contains:
one tube "Cofactor 1" (DTT);
one tube “Cofactor 2" (NAD);
one tube "Bacterial IMPDH" ;
one tube "Reaction buffer 10x" (1mL, contains hypoxanthine);
one tube of "Human Recombinant HPRT" for preparing enzyme solution at 75mU/ml(94.6nmol/h/ml);
one transparent 96-well plate.

Materials Required but Not Supplied

1) Plate agitator;
2) Plate reader fitted with a filter 340nm; Epoch; PerkinElmer;
3) PRPP (a-D-5-phosphoribosyl-1-pyrophosphate).
Important: PRPP is highly unstable once dissolved. We recommend to prepare the tubes with inducated mg of PRPP, store them as a powder at -20°C and dissolve it at very last moment.

Preparation

1.Preparation of hemolysates

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This protocol was developed with erythrocytes purified from 1mL of peripheral blood using FicollHypaque gradient and washed once with PBS. The pellet of PBS-washed packed erythrocytes (from 1ml of blood) was resuspended in 4mL of ice-cold dH20 and sonicated for 1min on ice (Sonopuls, Bandelin, 20% cycle, 50% power). The sonicated hemolysates were immediately used for HPRT measurement without additional centrifugation. The hemolysates can be also prepared by numerous freeze-thawing of erythrocytes resuspended in water and high speed centrifugation. Since the efficiency of hemolysis and release of HPRT enzyme depends on the method used for RBC disruption, we recommend to use always the same protocol of hemolysate preparation.
2. Preparation of 10ml "Reaction mixture 1x" (for performing 48 assays of 200µL, 24 with and 24 without PRPP)
1). Add 250µL of of deionized water to the tube with "Recombinant IMPDH". Agitate gently until complete dissolution of the powder.
2). Label a clean 15-ml tube "Reaction mixture 1x", transfer 1ml of "Reaction buffer 10x", followed by addition of 9 ml of deionized water.
3). Add the content of 2 tubes with "Cofactor 1", Cofactor 2" and "Bacterial IMPDH" to a 15-ml tube "Reaction mixture 1x".
To do so:
-pipet 1ml of buffer from "Reaction mixture 1x" to each of 2 tubes and mix them by inverting or pipeting up and down until the powder dissolved.
- transfer by pipeting the content of two tubes back into a vial "Reaction mixture 1x".
- 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.
4). Weight 5mg of PRPP in a clean labeled tube (15ml), add 5ml of prepared "Reaction mixture 1x". You have prepared: 5ml of "Reaction mixture 1x" (without PRPP, Blank); 5ml of "Reaction mixture 1x" with 2mM PRPP.

Assay Protocol

1. Positive control. Add indicated volume of deionized water to lyophilized human recombinant HPRT enzyme to provide 75mU/ml solution and mix gently until the powder is dissolved. Add 4µL of HPRT enzyme per well.
2. Add 4µL* of hemolysates per well.

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*Since the hemolysates show inherent optical density (OD) at 340nm, we strongly recommend to check the initial density of diluted hemolysates at 340nm before starting HPRT quantification. To do it, add 2, 4, or 6µL of hemolysates to the wells of 96-well plate followed by the addition of deionized water (qsp 200µL). Agitate for 2min and read the absorbance at 340nm. Use the volume of hemolysates providing OD in the range from 0.9 to 1.1 (usually it corresponds to 4µL of hemolysates per well).

3. Add 200µL of "Reaction mixture 1x without PRPP" (Blank) per well and 200µL of "Reaction mixture 1x" with 2mM PRPP.

4. Program plate reader for kinetics absorbance reading (every 5 min), 37°C.

Insert the plate into the reader pre-heated at 37°C, agitate for 2min and monitor the reaction at 340nm at 37°C for 2h with data collection every 5min.

Analysis

A. Calculation of activity of recombinant HPRT

1. For first two hours, calculate the absorbance rate per hour for reaction buffers with 2mM PRPP (ARPRPP) and without PRPP
(ARblank).

2. Calculate Mean ARPRPP and Mean ARblank

3. Calculate HPRT activity as follows:

HPRT Activity (in nmol/ml/hour)= (ARPRPP - ARblank)/#.1X106=
(0.500-0.037)/6220.0.789X106=94.6nmol/ml/h.

where:# is the molar extinction coefficient of NADH at 340nm:#=6200M-l.cm-l

l is the path-length = 0.789 for a 200µL-round-bottom well of 96-well microplate.

B. Calculation of HPRT activity in hemolysates

1. For first two hours, calculate the absorbance rate per hour for reaction buffers with 2mM PRPP (ARPRPP) and without PRPP (ARblanc). Calculate Mean ARPRPP 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. HPRT activity is calculated by the following formula:

Activity (in nmol / mg of hemoglobine per hour)= (Mean ARPRPP-Mean ARblank)/#.1.[Hgb]X106=

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84.62 nmol/mg of Hgb/h.
where: Mean ARPRPP = 0.490; Mean ARblank = 0.033.
is the molar extinction coefficient of NADH at 340nm:#=620M-l.cm-l.
l is the path-length: l=0.789 for a 200µL round-bottom well of 96-well microplate.
[Hgb], final haemoglobin concentration used in assay = 1.1mg/ml.

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