CREATIVE BIOMART®
Assay Kit

Continuous AMP Deaminase Assay Kit

Product Information

Cat.No. Kit-0875

Product Overview

AMP Deaminase Assay Kit is the first non-radioactive and continuous assay designed to measure AMP-deaminase content in samples. This enzymatic assay is based on a reaction involving Inosine Monophosphate Dehydrogenase (IMPDH).
(1) In the presence of AMP, AMP Deaminase (AMPD) 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 AMP deaminase activity in vitro or in cell lysates. For maximal accuracy, the assays with cell lysates are run with and without AMP substrate in parallel. The absorbance rate observed in the absence of P-ribose is used as blank and is subtracted from the absorbance rate measured in its presence.

Storage

The kit is shipped at room temperature since 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 assays (8 samples in triplicate)

Kit Components

Kit Contents (for 10mL of reaction mixture):
Once dissolved, the reagents can be stored at -20°C for three months and used to prepare as much of reaction mixture as needed.
A standard AMPD Assay Kit contains:
- Cysteine (10mg, powder);
- NAD (17mg, powder);
- AMP (12,5mg, powder);
- IMPDH enzyme, lyophilized;

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- Reaction buffer (glass vial, 10mL);
- one transparent 96-well plate (round-bottom 96-well plate)

Materials Required but Not Supplied

1) Plate agitator
2) Plate reader fitted with a filter 340nm (ex. Labsystems iEMS Reader MF (Thermo), Epoch (BioTec); PerkinElmer.

Assay Protocol

Preparation of "Reaction mixture" for one sample in duplicate
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 37°C;
3. Add 200µL of deionized water to the tube with "IMPDH enzyme", agitate (do not vortex to avoid foam), spin shortly;
4. Add 100µL of deionized water to each of three tubes (Cysteine, NAD and AMP). Vortex until complete dissolution, spin shortly;
5. To realise one assay in duplicate:
Add 0.85ml of reaction buffer in a clean 1.5mL tube,
9µL of cysteine
9µL of NAD.
Do not add AMP solution;
6. Add 18µL of "IMPDH enzyme" solution, close and agitate by inverting, spin shortly;
Composition of reaction mixture: 100mM Tris-HCl, 100mM KCl, 12mMMgCl2, 5mM cysteine, 2.5mM NAD, IMPDH >30mU/ml, pH 8.0, start by AMP (4.5mM)

Preparation of hemolysates
This protocol was developed with erythrocytes purified from 1mL of peripheral blood. The pellet of PBS-washed packed erythrocytes (from 1ml of blood) was resuspended in 4.5mL of ice-cold dH20 and sonicated for 1min on ice (Sonopuls, Bandelin, 20% cycle, 50% power). The sonicated

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hemolysates were immediately used for AMP-DA 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.

Reaction monitoring

1. Program plate reader for kinetics absorbance reading every 1min, 37°C;
2. Add 4µL of hemolysates (or1-10mUnits of AMP-Deaminase from otyher source) per well, followed by addition of 200µL of "Reaction mixture";
3. Insert the plate into the reader pre-heated at 37°C, agitate for 1 min and incubate for 15 min;
4. To start the reaction, add 4µL of AMP solution to two wells shown in red (two others will be used as Blank), agitate and monitor the reaction at 340nm at 37°C for 30 min or 1h with data collection every minute. Typical results obtained with RBC lysates are shown on Table 1 / Figure 1.

Analysis

1. Calculate the absorbance rate per hour for reaction buffers with AMP (ARAMP) and without (AR blank).
2. Calculate Mean ARAMP and Mean AR blank
3. Measure the concentration of hemoglobin [Hgb] in hemolysates using Drabkin's reagent and calculate final [Hgb] concentration used in assay.
4. AMPD activity is calculated by the following formula:

Activity = (MeanARAMP-MeanARblank)/(4.9x[Hgb])x1000 = (0.948-0.023)/(4.9x0.95)x1000 = 199 nmol/hour/mg of Hgb

Where:

MeanARAMP=0.948;

MeanARblank=0.023;

[Hgb], final haemoglobin concentration used in assay=0.95mg/ml.

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