CREATIVE BIOMART®
Assay Kit

ADK Assay Kit

Product Information

Cat

Kit-0880

Common Name

ADK

Cat.No. Kit-0880

Product Overview

Once released outside the cell, adenosine, a naturally occurring ribonucleoside, reveals strong neuroprotective and anti-inflammatory properties. One of the strategies to increase extracellular adenosine consists in inhibiting adenosine kinase (ADK), a ubiquitous enzyme that catalyzes the transfer of γ-phosphate from ATP to the 5'-hydroxyl of adenosine generating AMP and ADP.
ADK Assay Kit is a non isotopic assay that allows a continuous monitoring of ADK activity at 340nm through the coupling of ADK-catalyzed phosphorylation of inosine to the oxidation of IMP by a recombinant IMP-dehydrogenase (IMPDH). The assay developed in microplate format allows a HTS search of novel ADK inhibitors.
ADK Assay Kit was validated with a known ADK inhibitor (A-134974, IC50=20nM) and meets the requirements of a convenient and reliable HTS assay (microplate format, "add-and measure", spectrophotometric continuous readout, Z-factor = 0.68).
ADK Assay Kit can be also used to evaluate the phosphorylation of novel nucleoside analogues. In the absence of nucleoside
competitor, adenosine kinase phosphorylates inosine resulting in the formation of IMP directly monitored by the formation kinetics of NADH2 catalyzed by IMP-dehydrogenase. In the presence of nucleoside competitor, the phosphorylation of inosine, poor ADK substrate, is inhibited and detected as a decrease in NADH2 formation.

Description

The enzyme adenosine kinase (AdK; EC 2.7.1.20) catalyzes the transfer of gamma-phosphate from Adenosine triphosphate (ATP) to adenosine (Ado) leading to formation of Adenosine monophosphate (AMP). In addition to its well-studied role in controlling the cellular concentration of

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Ado, AdK also plays an important role in the maintenance of methylation reactions.

Quality Control Test

Measurement of Z'Factor of ADK assay.
Determine the absorbance rate per minute for every negative controls (ARC-), positive controls (ARC+) and blanks (ARBlank).
Calculate ARC- and ARC+, respectively the means of ARC- measurements and of ARC+ measurements.
Calculate σC- and σC+, respectively the standard deviations of ARC- measurements and of ARC+ measurements.
Z'Factor is then calculated as:
Z' = 1- (3σc++3σc-)/| ARC+ – ARC-
|Z-factor for ADK screening assay = 1-(3*0.0000594+3*0.000064)//0.000523-0.009454/ = 0.958-excellent assay.

Storage

ADK Assay Kit must be stored at -20°C until used.

Size

1 plate (96 assays); 5 plates (5 x 96 assays).

Kit Components

A standard ADK Assay Kit (one 96-well plate) contains:
1. one tube "IMPDH "
2. one tube "Cofactor 1" (DTT);
3. one tube "Cofactor 2" (NAD);
4. one tube "Human recombinant Adenosine Kinase";
5. one tube "ATP";
6. one vial "Reaction Buffer 5x";
7. one tube "ADK inhibitor" (A-134974);
8. one tube "50mM inosine";
9. Transparent 96-well plate.

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Materials Required but Not Supplied

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

Preparation

Microplate preparation.
i) To assess the performance of the assay, it is strongly recommended to run, in parallel with the assays and on the same microplate, several controls:
Positive controls, add 2μL of 50μM A-134974 dissolved in DMSO per well. A-134974 is a validated ADK inhibitor.
Negative controls, 2μL of DMSO.
Blank assays, 2μL of DMSO, no inhibitor, but where the reaction was NOT started.
Controls (in triplicate) and Blank Assays (in duplicate) can be set up in columns 1 and 12.
ii) Add 2-5μL of a water or DMSO-dissolved compounds to be tested into the empty wells.

Assay Protocol

ADK enzyme is pre-icubated with the inhibitor in the presence of ATP, reaction started by inosine addition
1. Preparation of standard reaction buffer (1x)
i) Add the content of "Reaction Buffer 5x" vial (4ml) to 16ml of deionized water to prepare “Reaction Buffer 1x”.
ii) Transfer quantitatively the content of 3 tubes with "Cofactor 1" (DTT), "Cofactor 2" (NAD), "ATP"to the tube with "Reaction buffer 1x".
To do so:
i) - transfer the content of three tubes (powder) into a vial "Reaction buffer 1x";
ii) – to be sure that all reagent and enzymes of the small tubes and vial are recovered, add 1 mL of "Reaction buffer 1x" to each empty tube, close, agitate and transfer the content back into the vial "Reaction buffer 1x", mix by gently inverting until complete dissolution. Avoid bubbles.
iii) Solubilize the content of "IMPDH" tube by adding 1ml of "Reaction buffer 1x" with co-factors. Close, agitate and transfer the content tube back into a vial "Reaction buffer 1x".
iv) Solubilize the content of "Human ADK enzyme" tube by adding 1ml of complete "Reaction buffer

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1x" with co-factors and IMPDH, transfer by pipeting the content of the tube back into a vial "Reaction buffer 1x".

Following ADK activity in vitro
1. Pre-incubation phase (15')
i) Program the plate-reader in a kinetics mode with the measurements done every 1 minutes, absorbance at 340 nm, 37°C, agitation before the kinetics for 1 min, duration time 15min.
ii) Add 200μL of standard reaction buffer per well.
iii) Agitate and measure absorbance at 340nm (A340). Record this first set of data.
2. Start the reaction by Inosine addition and follow-up the reaction for 40min
i) Eject the plate from the plate-reader.
ii) Program the plate-reader in a kinetics mode with the measurements done every 1 minutes, absorbance at 340 nm, 37°C, agitation before the kinetics for 1 min, duration time 40min.
iii) Start the reaction by adding 10μL* of 50mM of "Inosine" per well.
iv) Place the plate in the plate-reader and start the measurements. Record second set of data.
Composition of complete reaction buffer: 100mM Tris-HCl pH 8.5, 250mM KCl, 10mM MgCl2, 2.5mM NAD, 2.75mM ATP, IMPDH 20mU/ml; human recombinant ADK 2.2mU/ml-reaction started by inosine (final concentration 2.5mM*).
* the concentration of inosine can be further optimized by testing 0.5-1.25mM concentrations corresponding to 2-5μLof 50mM inosine per well.

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