PRECICE® ADK Assay Kit

for Adenosine Kinase activity measurement in vitro and the screening of ADK inhibitors

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.

PRECICE® ADK Assay Kit is the first non isotopic assay that allows a continuous monitoring of ADK activity at 340nm through the coupling of ADK-catalyzed phosphorylation of inosine and the oxidation of IMP by a recombinant IMP-dehydrogenase (IMPDH).

NOVOCIB's **PRECICE® ADK Assay Kit** is a very simple and fast assay for measuring Adenosine Kinase activity *in vitro*. It was specifically developed for the screening of ADK inhibitors and was validated with a known ADK inhibitor (A-134974). IC_{50} can be easily determined using this assay kit which meets the requirements for a convenient and reliable HTS assay (microplate format, "add-and measure", spectrophotometric continuous readout, an excellent performance with a Z'-Factor = 0.68 [*]).

Principle

NOVOCIB's **PRECICE® ADK Assay Kit** is based on the use of inosine as a substrate of ADK and a coupled reaction involving a highly active IMPDH (Inosine Monophosphate Dehydrogenase) for a direct measurement of the inosine monophosphate (IMP) formed by ADK.

In the presence of inosine and ATP, ADK catalyses the phosphorylation of inosine to form IMP and ADP. IMP is then immediately oxidized to Xanthosine Monophosphate (XMP) by a highly active IMPDH in the presence of NAD, leading to $NADH_2$ formation.

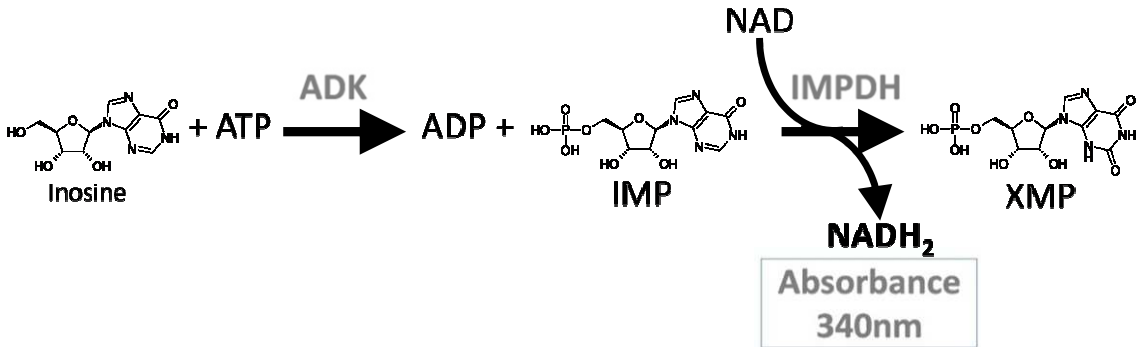

Figure 1: Principle of PRECICE® ADK Assay Kit for measuring Adenosine Kinase (ADK) activity

This coupling reaction is immediate when IMPDH activity is much higher than ADK activity in the assay. The enzymatic activity of ADK, which corresponds to the formation kinetics of IMP, is then stoichiometrically and directly monitored by the formation kinetics of $NADH_2$. The velocity of $NADH_2$ formation is measured with a spectrophotometer at 340nm (molar extinction coefficient of $NADH_2$ at 340nm = 6220 $M^{-1}.cm^{-1}$).

[*] **NOVOCIB** also provides Contract Research services for the screening of ADK inhibitors, according to the procedure described for PRECICE® ADK Assay Kit. In our conditions, the assay performance shows a Z'-Factor of 0.68, indicating an excellent assay performance. To know more, please refer to our **AK Inhibition Screening** services.

Validation

The use of **PRECICE® ADK Assay Kit** for the screening of ADK inhibitors and IC_{50} measurements was validated with compound A-134974, a known ADK inhibitor (Sigma-Aldrich, under license from Abbott Laboratories).

Z'-Factor was calculated from 44 negative (no ADK inhibition) and positive (inhibition of ADK with 500nM A-134974) and measured as 0.68, which is an excellent level of performance for a screening assay.

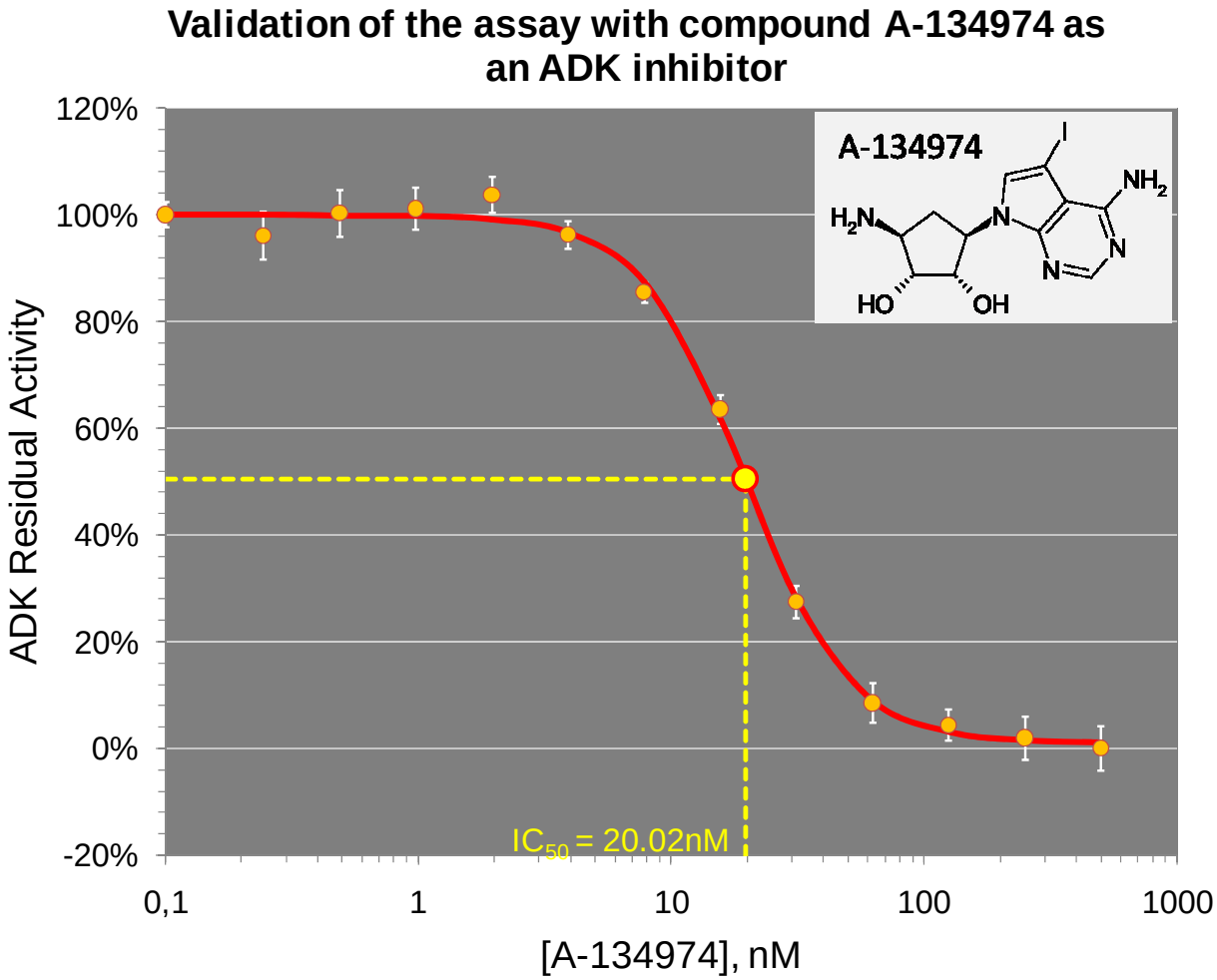

Figure 2: ADK Inhibition curve with compound A-134974 at 12 different concentrations.
All data points represent the mean (+/-SD) of 8 wells (2 wells - duplicate - on 4 independent plates). IC_{50} was determined by fitting a 4-parameter non-linear curve to the data with least-square method.
IMPDH activity assays were run to confirm that IMPDH is not inhibited by compound A-134974 (data not shown).

Kit Content:

- **Bacterial recombinant IMPDH, lyophilized**
- **IMPDH Reconstitution Buffer**
- **Human recombinant ADK, lyophilized**
- **ADK Reconstitution Buffer**
- **HKM Buffer (10x)**
- **NAD**
- **ATP**
- **DTT**
- **Inosine**

Related products & services:

- ***AK Inhibition Screening Services***
- ***Adenosine Kinase (AK), Human recombinant***
- ***Deoxycytidine kinase (dCK), Human recombinant***