Human adenosine kinase (AK)

Human, recombinant expressed in E.coli

EC 2.7.1.20

Synonyms: ADK, Adenosine 5'-phosphotransferase

Description

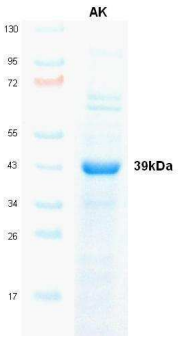
NOVOCIB's human adenosine kinase (AK) is a recombinant protein of *ca.39*kDa (345-aa short form[1, 2]) cloned by RT-PCR amplification of mRNA extracted from human hepatoma cells and expressed in *E.coli*. The sequence of the cloned AK (GenBank accession number U50196) was confirmed by DNA sequencing (100% identity).

Adenosine kinase is a ubiquitous enzyme that catalyzes the transfer of γ-phosphate from ATP to 5' hydroxyl of adenosine generating AMP and ADP. Adenosine (AR) is an important modulator of central nervous system functions with a half-life of seconds. Facilitated diffusion of adenosine across the cell membrane closely couples adenosine concentrations in the intracellular and extracellular compartments. Inhibition of adenosine kinase results in selective increase of local adenosine concentrations and reduced seizure susceptibility and nociception *in vivo*[3]. Adenosine kinase is an attractive and experimentally validated target for the development of new analgesic and anti-inflammatory agents[4]. In addition, AK has recently emerged as a novel target to predict and to prevent epileptogenesis[5, 6], to treat schizophrenia[7] or to limit brain injury after an ischemic stroke[8]. The X-ray crystallographic structure of human AK has been described[9] and provides structural basis for rational design and optimisation of new AK inhibitors.

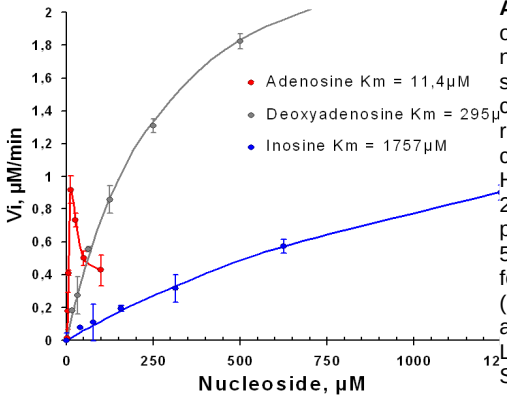
In addition, this enzyme is responsible for the phosphorylation and consequent clinical activity of several therapeutically useful nucleosides, including the antiviral drug ribavirin[10], immunosuppressive drug mizoribine[11] and anticancer C-nucleoside, tiazofurin[12].

Storage: –20 ℃ in a solution containing 50 mM Tris-HCl, pH 7.6, 1 mM β-mercaptoethanol, 50% glycerol.

Unit Definition: One unit of adenosine kinase converts 1.0 µmole of adenosine and ATP to AMP and ADP per minute at pH 7.6 at 30℃, as measured by a coupled PK/LDH enzyme system.



Specific Activity: ≥ 0.030 unit/mg protein.
Purity: controlled by 10% AA SDS-PAGE.



Assay condition: Enzymatic activity of adenosine kinase with particular nucleoside substrate is measured by spectrophotometric assays in a coupled lactate dehydrogenase / pyruvate kinase system. Assays were carried out at 37℃, at 50mM Tris-HCl pH7,6; 50mM KCl, 5mM MgCl2, 2,5mM ATP, 0,1mM NADH, 1mM phosphoenolpyruvate, 1mM DTT, PK 5U/ml, LDH 5U/ml. Reaction was followed in an iEMS Reader MF (Labsystems) microtiter plate reader at 340nm. Nucleosides, nucleotides, LDH and PK were purchased from Sigma-Aldrich.

Related products:

NOVOCIB has cloned and purified a panel of human recombinant nucleoside kinases and has developed a range of PRECICE® services to evaluate substrate properties of new nucleoside analogues for key cellular kinases.

- ***Adenosine kinase phosphorylation assay***
- ***Coupled Nucleoside Kinase – IMPDH II***
- ***Deoxycytidine kinase (dCK)***
- ***UMP-CMP kinase (CMK)***
- ***Cytosolic 5' nucleotidase II (cN-II)***
- ***CMK nucleotide monophosphate phosphorylation assay***
- ***dCK nucleoside phosphorylation assay***

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- ***Coupled dCK-CMK nucleoside phosphorylation assays***
- ***cN-II phosphorylation assay***

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