

Human adenosine kinase (AK) Human, recombinant expressed in *E.coli* EC 2.7.1.20

Synonyms: ADK, Adenosine 5'-phosphotransferase

Description

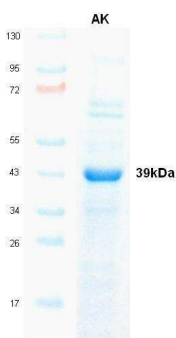
NOVO CIB's human adenosine kinase (AK) is a recombinant protein of ca.39kDa (345-aa short form^{1, 2}) cloned by RT-PCR amplification of mRNA extracted from human hepatoma cells and expressed in *E.coli*. The sequence of the cloned AK (GenBank accession number U50196) was confirmed by DNA sequencing (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 agents⁴. In addition, AK has recently emerged as a novel target to predict and to prevent epileptogenesis^{5, 6}, to treat schizophrenia⁷ or to limit brain injury after an ischemic stroke⁸. The X-ray crystallographic structure of human AK has been described⁹ and provides structural basis for rational design and optimisation of new AK inhibitors.

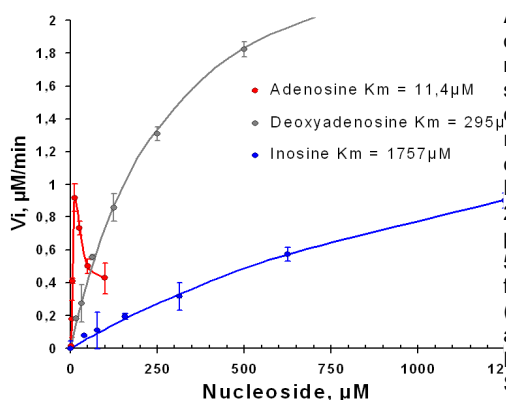
In addition, this enzyme is responsible for the phosphorylation and consequent clinical activity of several therapeutically useful nucleosides, including the antiviral drug ribavirin¹⁰, immunosuppressive drug mizoribine¹¹ and anticancer C-nucleoside, tiazofurin¹².

Storage: -20 °C 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 μ mole of adenosine and ATP to AMP and ADP per minute at pH 7.6 at 30°C, as measured by a coupled PK/LDH enzyme system.



Specific Activity: \geq 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°C, 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:

NOVO CIB 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

¹ Spychala J, Datta NS, Takabayashi K, Datta M, Fox IH, Gribbins T, Mitchell BS Cloning of human adenosine kinase cDNA: Sequence similarity to microbial ribokinases and fructokinases (1996) *Proc. Natl. Acad. Sci. USA* 93, pp. 1232-1237

² Sahin B, Kansy JW, Nairn AC, Spychala J, Ealick SE, Fienberg AA, Greene RW and Bibb JA. Molecular characterization of recombinant mouse adenosine kinase and evaluation as a target for protein phosphorylation *Eur. J. Biochem.* 271, 3547-3555 (2004)

³ Jarvis MF, Yu H, Kohlhaas K, Alexander K, Lee CH, Jiang M, Bhagwat SS, Williams M, Kowaluk EA ABT-702 (4-Amino-5-(3-bromophenyl)-7-(6-morpholinopyridin-3-yl)pyrido[2,3-d]pyrimidine), a Novel Orally Effective Adenosine Kinase Inhibitor with Analgesic and Anti-inflammatory Properties: I. In Vitro Characterization and Acute Antinociceptive Effects in the Mouse *Journal of Pharmacology and Experimental Therapeutics* 295:1156-1164, 2000

⁴ McGaraughty S, Cowart M, Jarvis MF, Berman RF. Anticonvulsant and antinociceptive actions of novel adenosine kinase inhibitors *Curr Top Med Chem.* 2005;5(1):43-58

⁵ Fedele DE, Gouder N, Guttinger M, Gabernet L, Scheurer L, Rulicke T, Crestani F, Boison D Astrogliosis in epilepsy leads to overexpression of adenosine kinase, resulting in seizure aggravation (2005) *Brain*, 128, 2383-2395

⁶ Detlev Boison The adenosine kinase hypothesis of epileptogenesis *Progress in Neurobiology* 84 (2008) 249-262

⁷ Boison D, Singer P, Shen HY, Feldon J, Yee BJ Adenosine hypothesis of schizophrenia – Opportunities for pharmacotherapy (2011), doi:10.1016/j.neuropharm.2011.01.048

⁸ Shen HY, Lusardi TA, Williams-Karnesky RL, Lan JQ, Poulsen DJ, Boison D Adenosine kinase determines the degree of brain injury after ischemic stroke in mice (2011) *J. Cereb. Blood Flow Metab.* 31(7), 1648-1659

⁹ Mathews, I.L., Erion, M.D. & Ealick, S.E. Structure of human adenosine kinase at 1.5 Å resolution. (1998) *Biochemistry* 37, 15607-15620

¹⁰ Willis RC, Carson DA, Seegmiller JE. Adenosine Kinase Initiates the Major Route of Ribavirin Activation in a Cultured Human Cell (1978) *PNAS* 1978;75:3042-3044

¹¹ Miller RL, Adamczyk DL, Miller WH, Koszalka GW, Rideout JL, Beacham LM, Chao EY, Haggerty JJ, Krenitsky TA, Elion, GB Adenosine kinase from rabbit liver II Substrate and inhibitor specificity. (1979) *J. Biol. Chem.* 254, 2346-2352

¹² Saunders PP, Spindler CD, Tan MT, Alvarez E, Robins RK Tiazofurin Is Phosphorylated by Three Enzymes from Chinese Hamster Ovary Cells (1990) *Cancer Research* 50, 5269-5274



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