Human deoxycytidine kinase (dCK)

Human, recombinant expressed in E.coli

E.C. 2.7.1.74

Description

NOVOCIB's human deoxycytidine kinase (dCK) is a recombinant protein of *ca.33*kDa cloned by RT-PCR amplification of mRNA extracted from human hepatoma cells and expressed in *E.coli*.

Human deoxycytidine kinase plays a key role in the salvage pathway of deoxynucleotides synthesis providing resting cells with deoxynucleotides for DNA repair and mitochondrial DNA synthesis. The enzyme has a broad substrate specificity and provides the phosphorylation of both purine and pyrimidine deoxynucleosides (*e.g.* deoxyadenosine (dA), deoxyguanosine (dG)) and deoxycytidine (dC) and pyrimidine ribonucleoside, cytidine (C)). The enzyme can utilize both ATP and UTP as phosphate donor with UTP as a preferred substrate.

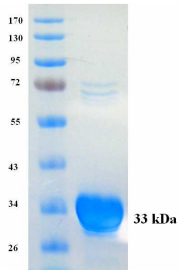
Deoxycytidine kinase is responsible for the phosphorylation and activation of numerous nucleoside analogs used to treat cancer (*e.g.* cytarabine, gemcitabine, cladribine and fludarabine) including nucleoside analogs of non-physiological L-chirality (*e.g.* 3TC, lamivudine, anti-HIV and anti-hepatitis B agent). Three-dimensional structures of dCK in complex with various pyrimidine[1] and purine[2, 3] D- and L-nucleosides have been solved providing structural basis for activation of L- and D-nucleoside analogs.

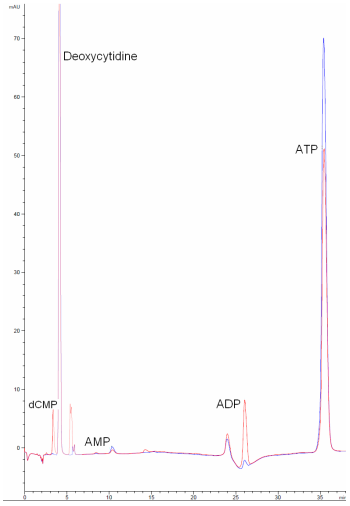
Storage: –20 ℃ in a solution containing 50 mM Tris-HCl, pH 7.6, 1 mM β-mercaptoethanol, 50% glycerol.

Unit Definition: One unit of deoxycytidine kinase converts 1.0 µmole of deoxycytidine and ATP to dCMP and ADP per minute at pH 7.6 at 37℃, as measured by a coupled PK/LDH enzyme system.

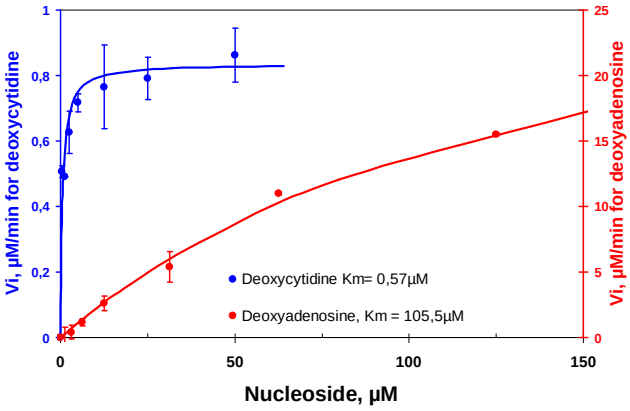
Specific Activity: ≥ 0.025 unit/mg protein.

Purity: controlled by 12%AA SDS-PAGE.





The enzymatic activity of human recombinant dCK was confirmed by ion-pair HPLC analysis (Agilent 1100 series, Zorbax C18plus) as shown by formation of dCMP and ADP (red) from deoxycytidine and ATP (blue).



Assay condition: Enzymatic activity of dCK 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, 10mM MgCl2, 5mM ATP, 0,1mM NADH, 1mM phosphoenolpyruvate, 1mM DTT, PK 10U/ml, LDH 15U/ml, 0,9µM dCK. 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.

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

[1] Sabini E, Ort S, Monnerjahn C, Konrad M, Lavie A. **Structure of human dCK suggests strategies to improve anticancer and antiviral therapy.** (2003) *Nat Struct Biol*.10(7):513-9
[2] Sabini E, Hazra S, Ort S, Konrad M, Lavie A. **Structural basis for substrate promiscuity of dCK**. (2008) *J Mol Biol.* 378(3):607-21
[3] Sabini E, Hazra S, Konrad M, Burley SK, Lavie A. (2007) **Structural basis for activation of the therapeutic L-nucleoside analogs 3TC and troxacitabine by human deoxycytidine kinase**. *Nucleic Acids Res*. 35(1):186-92