Nucleoside kinases, rate-limiting step of nucleoside analogues activation

Nucleoside analogues have proven to be a highly successful class of anti-cancer and anti-viral drugs. The therapeutic efficacy of nucleoside analogues is dependent of their intracellular phosphorylation. Two cellular nucleoside kinases, deoxycytidine kinase (dCK) and UMP-CMP kinase (CMK) are critical for phosphorylation of cytidine analogues. These kinases provide two first steps of activation of highly effective anti-cancer and anti-viral drugs, such as 1-β-D-arabinofuranosylcytosine (araC, **aracytidine**), 2',2'difluorodeoxycytidine (dFdC, **gemcitabine**), β-D-2'3'-dideoxycytidine (ddC). Both kinases phosphorylate unnatural L-nucleosides (e.g., β-L-2'3'-dideoxy-3'thiacytidine, L-SSdC, 3-TC or **lamividune**). Kinetic constants of araC, dFdC and 3TC phosphorylation by recombinant dCK and UMP-CMPK have been published. The comparison of phosphorylation properties of new nucleoside analogues with those of known drugs provides the rational basis for selection of analogues of better therapeutic potential.
To characterize the phosphorylation properties of new nucleoside analogues, **NovoCIB** has developed human recombinant dCK and human recombinant CMK nucleoside phosphorylation assays. As shown in Table 1, CMK assay must be performed with monophosphate forms of nucleoside analogues and requires preliminary phosphorylation of nucleoside analogues and their purification. To circumvent this time-consuming step, **NovoCIB** has developed a coupled dCK-CMK nucleoside phosphorylation assay that delivers in one step the critical information on both dCK and CMK substrate properties of nucleoside analogue.

Ribavirin (1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxamide) is a purine nucleoside analogue with a broad-spectrum antiviral activity. Since the 1970's[1], it is known that the initial step of ribavirin phosphorylation is provided by adenosine kinase. Recently it has been demonstrated that cytosolic 5'-nucleotidase II can also phosphorylate ribavirin, that could contribute to the development of ribavirin-induced haemolytic anemia *in vivo*[2]. **NovoCIB** has developed both human recombinant adenosine kinase and cytosolic nucleotidase II nucleoside phosphorylation assays to evaluate the properties of new ribonucleoside analogues in comparison with those of ribavirin.

	dCK assay	CMK assay	Coupled dCK-CMK assay	AK assay	5'cN-II assay
Natural substrates	Deoxyadenosine Deoxyguanosine Deoxycytidine Cytidine	dCMP, UMP CMP	Deoxycytidine Cytidine	Adenosine Inosine	Deoxyinosine Inosine
Nucleoside analogues substrates	Cladribine, fludarabine Gemcitabine, Lamivudine, Aracytidine Fluorodeoxyuridine	**Monophosphate forms** of cytidine analogues dFdCMP, araCMP, 3TCMP Adefovir (9-(2-phosphonomethoxyethyl) adenine	Gemcitabine Aracytidine Lamivudine Fluodeoxyuridine	Ribavirin Tubercidin Mizoribin	Dideoxyinosine Ribavirin Acyclovir

Table 1. Available nucleoside kinase assays and reference nucleoside substrates

[1] R. C. Willis, D. A. Carson, and J. E. Seegmiller (1978**) Adenosine Kinase Initiates the Major Route of Ribavirin Activation in a Cultured Human Cell Line.** *PNAS* USA, 75: 3042-3044
[2] Wu JZ, Larson G, Walker H, Shim JH, Hong Z. Phosphorylation of ribavirin and viramidine by adenosine kinase and cytosolic 5'-nucleotidase II: Implications for ribavirin metabolism in erythrocytes (2005) Antimicrob Agents Chemother. 49(6):2164-71.

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*.

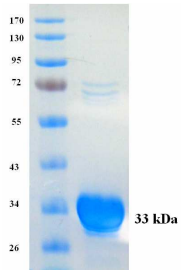
Human deoxycytidine kinase plays a key role in the salvage pathway of deoxynucleotides synthesis providing resting cells with deoxynucleotides for DNA repair and mitochondrial DNA synthesis. The enzyme has a broad substrate specificity and provides the phosphorylation of both purine and pyrimidine deoxynucleosides (*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.

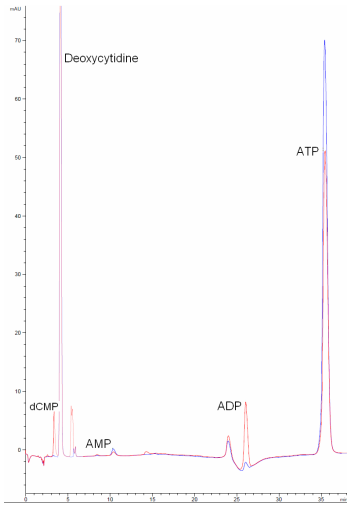
Deoxycytidine kinase is responsible for the phosphorylation and activation of numerous nucleoside analogs used to treat cancer (*e.g.* cytarabine, gemcitabine, cladribine and fludarabine) including nucleoside analogs of non-physiological L-chirality (*e.g.* 3TC, lamivudine, anti-HIV and anti-hepatitis 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.

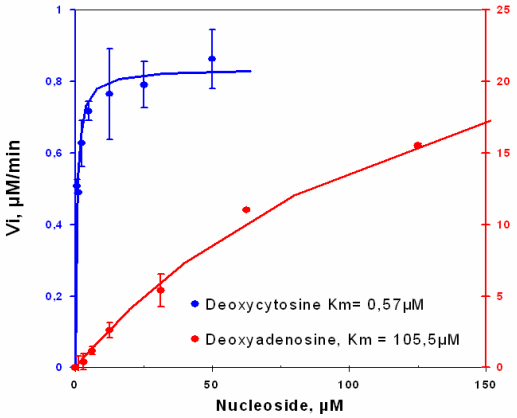
Unit Definition: One unit of deoxycytidine kinase converts 1.0 µmole of deoxycytidine and ATP to dCMP and ADP per minute at pH 7.6 at 37℃, as measured by a coupled PK/LDH enzyme system.

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:

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- ***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

dCK nucleoside phosphorylation assay

IMPORTANT: Client-specified alterations can be accommodated

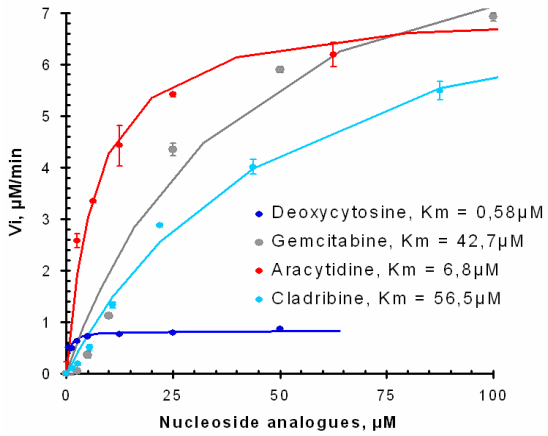
Aim: Characterization of substrate properties (Km and Vmax) of new nucleoside analogues for human deoxycytidine kinase in comparison with the properties of known nucleoside analogues (*e.g.* **aracytidine, gemcitabine, cladribine and lamivudine**).

Substrate	Novocib*			Published data		Ref.
	Km,µM	Vmax, µmol/mg/min	Relative Vmax, % of dCR	Km,µM	Vmax, µmol/mg/min	
Deoxycytidine	**0,577**	**0,026**	**100**	0,16	0,033	Recombinant Johansson Karlsson 1995[1]
				1,3	0,069	Recombinan Usova & Eriksson, 1997 t[2]
				0,57	0,004	Partially purified Someya H et al 2003[3]
Gemcitabine	**42,71**	**0,325**	**1250**			
Deoxyadenosine	**150,5**	**1,08**	**4153**	115		Recombinant Sabini E et al 2008[4]
				480	1,5	Recombinant Johansson Karlsson 1995
Aracytidine	**6,81**	**0,224**	**862**	15	0,009	Partially purified Someya H et al 2003
Cladribine	**56,5**	**0,285**	**1096**	89	0,126	Recombinant Usova & Eriksson, 1997
				24	0,76	Recombinant Johansson Karlsson 1995

Enzyme: The dCK used in the assays is a human recombinant dCK, cloned from human cells, expressed in *E. coli*, produced and purified by **NOVOCIB** (see sheet # E-Nov 3 for further information). The enzyme purity is controlled by SDS-PAGE. Protein concentration is measured by Bradford method (Bio-Rad). dCK enzymatic activity (≥ 0.025 unit/mg protein) is systematically controlled before performing any assay.

Kinetics Analysis: Enzymatic activity of deoxycytidine kinase with particular nucleoside substrate is measured continuously by spectrophotometric assays in a coupled lactate dehydrogenase/pyruvate kinase system. Assays are carried out at 37°C, at 50mM Tris-HCl pH7,6; 50 mM KCl, 10mM MgCl2, 5mM ATP, 0,1mM NADH, 1mM phosphoenolpyruvate, 1mM DTT, PK 10U/ml, LDH 15U/ml, 0,9µM dCK. The nucleosides, nucleotides, LDH and PK are purchased from Sigma-Aldrich. Reaction is followed in an iEMS Reader MF (Labsystems) microtiter plate reader at 340nm. Assays are performed in duplicate (2 wells per compound and per concentration). Triplicates are available upon request. Km and Vmax are calculated from spectroscopic data using Michaelis-Menten equation.

A confirmation by HPLC analysis of formation of monophosphorylated forms is available upon request.



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- ***Adenosine kinase phosphorylation assay***
- ***cN-II phosphorylation assay***

[1] M. Johansson and A. Karlsson (1995): **Differences in kinetic properties of pure recombinant human and mouse deoxycytidine kinase** *Biochem. Pharmacol.* 50(2), 163-168
[2] E. V. Usova and S. Eriksson (1997) **The effects of high salt concentrations on the regulation of the substrate specificity of human recombinant deoxycytidine kinase** *Eur. J. Biochem.* 248(3), 762-766
[3] H. Someya *et al.* (2003) **Phosphorylation of 4'-thio-beta-D-arabinofuranosylcytosine and its analogs by human deoxycytidine kinase** *J. Pharmacol. Exp. Ther.* 304(3), 1314-1322
[4] E. Sabini *et al.* (2008) **Structural basis for substrate promiscuity of dCK** *J. Mol. Biol.* 378(3), 607-621

UMP-CMP kinase (CMK)

Human, recombinant expressed in E.coli

E.C. 2.7.4.14

Synonyms: cytidylate kinase, deoxycytidylate kinase, deoxycytidine monophosphokinase, dCMP kinase, cytidine monophosphate kinase, CMP kinase (CMK, CMPK), uridine monophosphate kinase (UMK, UMPK), uridine monophosphate/cytidine monophosphate kinase, UMP/CMP kinase (UMP/CMPK), CTP:CMP phosphotranferase, ATP:UMP-CMP phosphotransferase, pyrimidine nucleoside monophosphate kinase (YMPK)

Description

NOVOCIB's Human UMP-CMP kinase (CMK) is a recombinant protein of *ca.* 27kDa (full length 228-aa form[1]) cloned by RT-PCR amplification of mRNA extracted from Huh7 cells (human hepatoma) and expressed in *E.coli*.

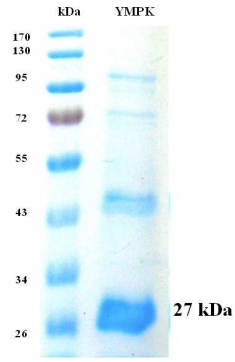
UMP-CMP kinase plays a critical role in supplying cells with nucleotides by catalysing the phosphorylation of CMP, UMP and dCMP to their respective diphosphates. CMK plays also an important role in the activation of cytidine analogues, aracytidine and gemcitabine, a mainstay of leukaemia and lymphoma therapy[2]. CMK has a remarkable ability of to phosphorylate L-nucleotides from their monophosphate to diphosphate forms[3] as shown for β-L-2'3'-dideoxy-3'thiacytidine (L-SSdC, 3-TC or lamividune), an anti-HIV and anti-hepatitis B drug.

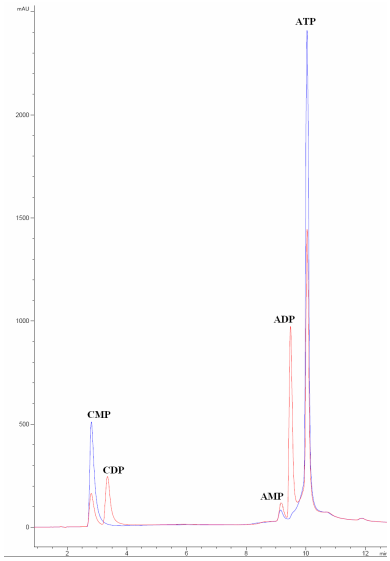
Crystal structure of open form of human UMP-CMP kinase has been solved recently[4]. These data, together with the homology model of enzyme in closed state, provides structural basis for understanding the substrate specificity of the enzyme and helps to design new nucleoside analogues of higher phosphorylation efficiency.

Storage: –20 ℃ in a solution containing 150mM KCl, 50mM Tris-Hcl, pH7,5, 2mM β-mercaptotethanol, 50% glycerol.

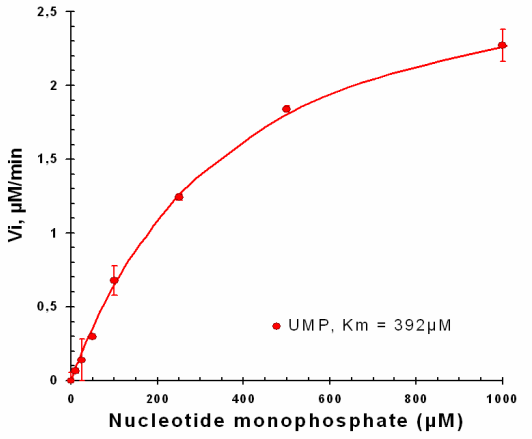
Unit Definition: One unit of UMP-CMP kinase converts 1.0 µmole of UMP and ATP to UDP and ADP per minute at pH 7.6 at 25℃, using a coupled enzyme system with PK/LDH.

Specific activity: ≥0,150U/mg
Purity: controlled by SDS-PAGE





The enzymatic activity of recombinant UMP-CMP kinase was confirmed by HPLC analysis as illustrated by CDP and ADP formation (red) from CMP and ATP (blue).



Assay condition: Enzymatic activity of UMP-CMP kinase is measured by continuous spectrophotometric assays in a coupled lactate dehydrogenase/pyruvate kinase system. Assays are carried out at 37℃, at 5 0mM Tris-HCl pH7,6; 50mM KCl, 10mM $MgCl_2$, 5mM ATP, 0,1mM NADH, 1mM phosphoenolpyruvate, 1mM DTT, PK 10U/ml, LDH 15U/ml, 380nM CMK. Reaction is followed in an iEMS Reader MF (Labsystems, Finland) microtiter plate reader at 340nm.

Related products:

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- ***Adenosine kinase phosphorylation assay***
- ***cN-II phosphorylation assay***
- ***Coupled Nucleoside Kinase – IMPDH II***

[1] Jieh-Yuan Liou, Ginger E. Dutschman, Wing Lam, Zaoli Jiang and Yung-Chi Cheng (March 2002) **Characterization of Human UMP/CMP Kinase and Its Phosphorylation of D- and L-Form Deoxycytidine Analogue Monophosphates** *Cancer Research* 62, 1624-1631
[2] Van Rompay AR, Johansson M, and Karlsson A (September 1999) **Phosphorylation of Deoxycytidine Analog Monophosphates by UMP-CMP Kinase: Molecular Characterization of the Human Enzyme** *Mol Pharmacol* 56 (3), 562-569
[3] Claudia Pasti, Sarah Gallois-Montbrun, Hélène Munier-Lehmann, Michel Veron, Anne-Marie Gilles and Dominique Deville-Bonne (Mar2003) **Reaction of human UMP-CMP kinase with natural and analog substrates**. *European Journal of Biochemistry*, 270 (8), 1784 - 1790
[4] Segura-Peña D, Sekulic N, Ort S, Konrad M, Lavie A. (2004) **Substrate-induced conformational changes in human UMP/CMP kinase**. *J Biol Chem.* 279(32):33882-33889

CMK nucleotide monophosphate phosphorylation assay

IMPORTANT: Client-specified alterations can be accommodated.

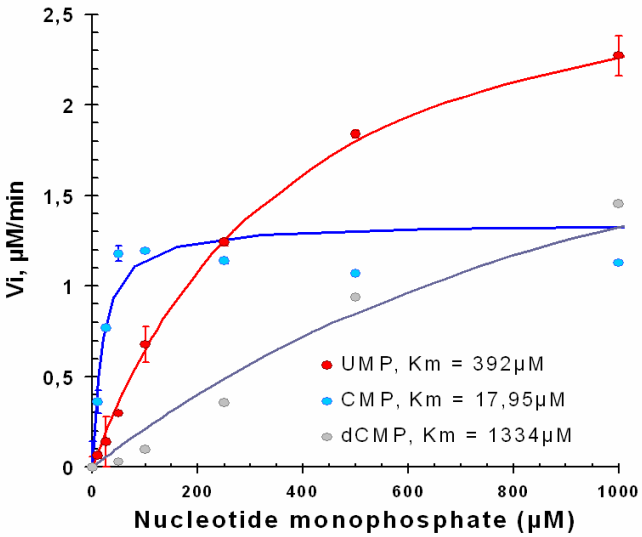
Aim: Characterization of substrate properties (Km and Vmax) of monophosphate forms of new nucleoside analogues for human CMK in comparison with monophosphate forms of natural nucleosides or reference nucleoside analogues.

Enzyme: The enzyme used in the assays is a human recombinant CMK, cloned from human cells, expressed in *E. coli*, produced and purified by **NOVOCIB** (see sheet # E-Nov 4 for further information). The enzyme purity is controlled by SDS-PAGE. Protein concentration is measured by Bradford method (Bio-Rad). CMK enzymatic activity (≥ 0.150 unit/mg protein) is systematically controlled before performing any assay.

			NovoCIB*	Published data[6]	
	Km, µM	***Vmax* nmol/mg/min**	***Relative Vmax, % of CMP***	*Km, µM*	*Vmax,* nmol/mg/min
CMP	**17.9**	**130.07**	**100**	20	350
UMP	**392**	**307.16**	**236**	45	350
dCMP	**1334**	**297.65**	**228**	900	200

Kinetics Analysis: Substrate properties of a particular nucleoside monophosphate for CMK are evaluated in a continuous LDH/PK spectrophotometric assay. The assays are carried out at 37°C, at 50mM Tris-HCl pH 7.6; 50mM KCl, 10mM $MgCl_2$, 5mM ATP, 0.1mM NADH, 1mM phosphoenolpyruvate, 1mM DTT, PK 10U/ml, LDH 15U/ml, 380nM CMK. Nucleosides, nucleotides, LDH and PK are purchased from Sigma-Aldrich. Reaction is followed in an iEMS Reader MF (Labsystems) microtiter plate reader at 340nm. Assays are performed in duplicate (2 wells per compound and per concentration). Triplicates are available upon request. Km and Vmax are calculated from spectroscopic data using Michaelis-Menten equation.

A confirmation by HPLC analysis of the formation of monophosphorylated forms is available upon request.



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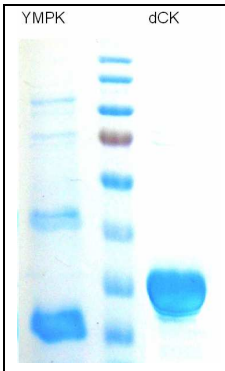
- ***Adenosine kinase phosphorylation assay***
- ***cN-II phosphorylation assay***
- ***Coupled Nucleoside Kinase – IMPDH II***

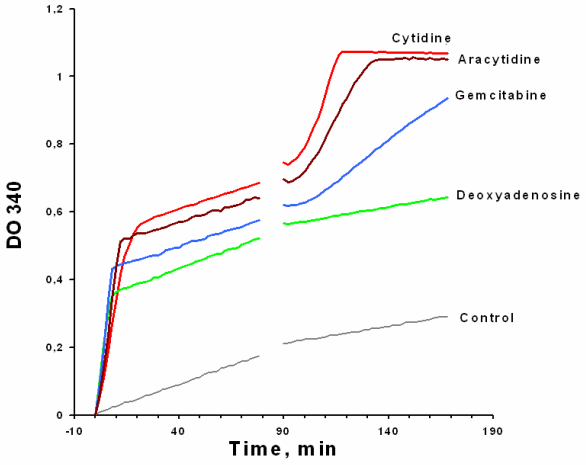
References
Chih-Hung Hsu, Jieh-Yuan Liou, Ginger E. Dutschman, and Yung-Chi Cheng (2005) **Phosphorylation of Cytidine, Deoxycytidine, and Their Analog Monophosphates by Human UMP/CMP Kinase Is Differentially Regulated by ATP and Magnesium** *Mol Pharmacol* 67:806-814
Topalis D, Kumamoto H, Amaya Velasco MF, Dugué L, Haouz A, Alexandre JA, Gallois-Montbrun S, Alzari PM, Pochet S, Agrofoglio LA, Deville-Bonne D. (Jul 2007) **Nucleotide binding to human UMP-CMP kinase using fluorescent derivatives – a screening based on affinity for the UMP-CMP binding site**. *FEBS J.* 274(14):3704-14

Coupled dCK-CMK nucleoside phosphorylation assays

Aim: Coupled dCK-CMK nucleoside phosphorylation assay is a cost-effective rapid assay that delivers in one step the critical information on both dCK and CMK substrate properties of nucleoside analogue.

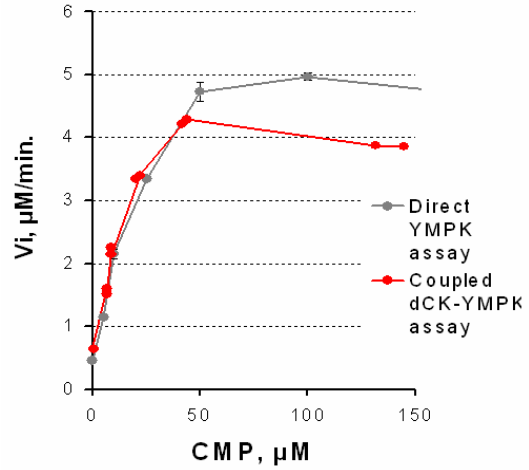


Enzymes: The enzymes used in this assay are human recombinant dCK and human recombinant CMK, cloned by NovoCIB from human cells, expressed in *E. coli*, and produced and purified by **NOVOCIB** (see sheet # E-Nov **3** and # E-Nov 4 for detailed information).



Assay condition:

Substrate properties of nucleoside analogue for dCK and CMK kinases are evaluated in a two-step spectrophotometric assay carried out at 37°C, at 50mM Tris-HCl pH7,6; 50mM KCl, 10mM $MgCl_2$, 5mM ATP, 0.1mM NADH, 1mM phosphoenolpyruvate, 1mM DTT, PK 10U/ml, LDH 15U/ml. The initial phosphorylation is started by addition of dCK (1µM) and the reaction is followed spectropho-metrically at 340nm during 90 min followed by addition of CMK (0.3µM). The changes in absorbance at 340nm are used to calculate both the initial rate of reactions and the concentration of nucleoside mono-phosphate formed.



Method validation :

The phosphorylation kinetic of CMP by recombinant CMK have been measured in two independent approaches. In first one, CMK Km for CMP was studied directly with CMP substrate (grey), and in second one CMK Km for CMP was measured indirectly in coupled dCK-CMK assay (red) usine cytidine as a substrate. As shown on left, coupled dCK-CMK assay produces results which are highly similar to those obtained from a direct CMK assay.

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- ***Cytosolic 5' nucleotidase II (cN-II)***
- ***Adenosine kinase phosphorylation assay***
- ***cN-II phosphorylation assay***
- ***Coupled Nucleoside Kinase – IMPDH II***

Human adenosine kinase (AK)

Human, recombinant expressed in E.coli

EC 2.7.1.20

Synonyms: ADK, Adenosine 5'-phosphotransferase

Description

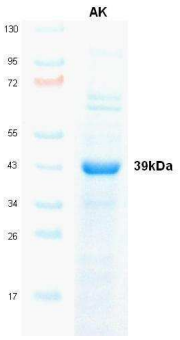
NOVOCIB's human adenosine kinase (AK) is a recombinant protein of *ca.39*kDa (345-aa short form[1, 2]) cloned by RT-PCR amplification of mRNA extracted from human hepatoma cells and expressed in *E.coli*. The sequence of the cloned AK (GenBank accession number U50196) was confirmed by DNA sequencing (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 recently has emerged as a novel target to predict and to prevent epileptogenesis[5, 6]. The X-ray crystallographic structure of human AK has been described[7] and provides structural basis for rational design and optimisation of new AK inhibitors.

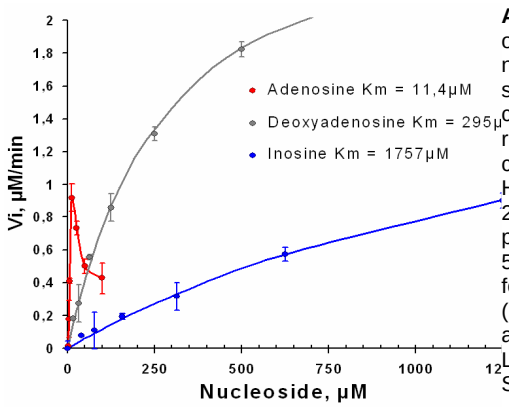
In addition, this enzyme is responsible for the phosphorylation and consequent clinical activity of several therapeutically useful nucleosides, including the antiviral drug ribavirin[8], immunosuppressive drug mizoribine[9] and anticancer C-nucleoside, tiazofurin[10].

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.

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

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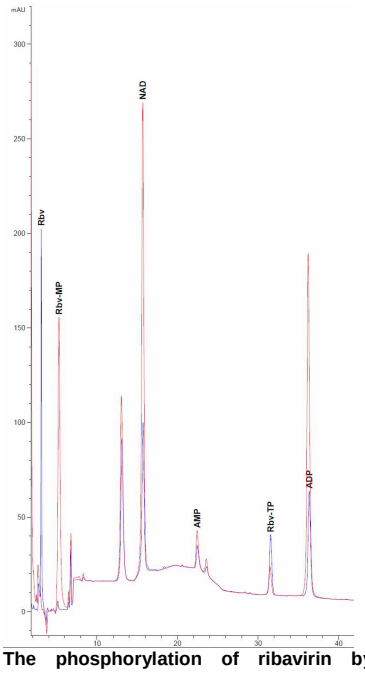
AK nucleoside phosphorylation assay

IMPORTANT: Client-specified alterations can be accommodated.

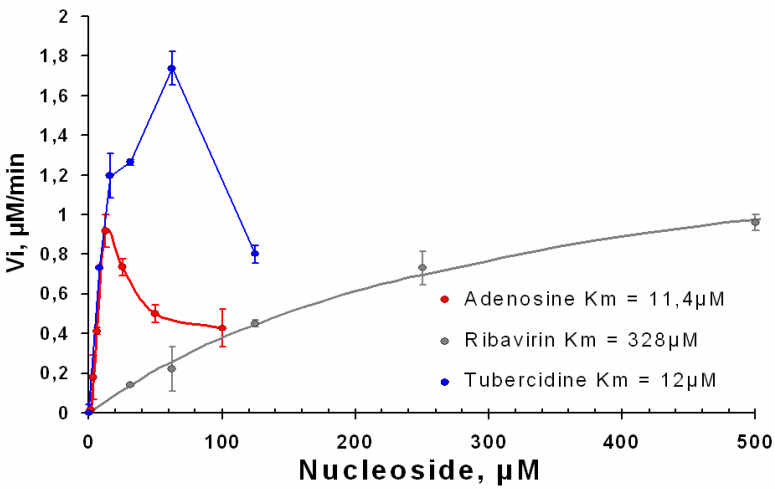
Aim: Characterization of substrate properties (Km and Vmax) of new nucleoside analogues for human adenosine kinase in comparison with properties of known nucleoside analogues (*e.g.* **ribavirine, tubercidine or mizoribine**).

Substrate	Novocib		Published		
	Km (µM)	Kcat (min^{-1})	Km (µM)	Kcat (min^{-1})	Ref
Adenosine	11	1.5	3.2	13	1
			0.150		2
Ribavirine	328	1,9	540	1,8	1
Deoxyadenosine	295	3,4	360		2
Tubercidine	12	2,2			
Inosine	1758	2,6			

Enzyme: The AK used in the assays is a human recombinant AK, cloned from human cells, expressed in *E. coli*, produced and purified by **NOVOCIB** (see sheet # E-Nov5 for further information). The enzyme purity is controlled by SDS-PAGE. Protein concentration is measured by Bradford method (Bio-Rad). AK enzymatic activity (≥ 0.030 unit/mg protein) is systematically controlled before performing any assay.



The phosphorylation of ribavirin by adenosine kinase was confirmed by HPLC analysis as illustrated by Ribavirine-MP formation (red) from ribavirine (blue).



Kinetics Analysis: Enzymatic activity of adenosine kinase with particular nucleoside substrate is measured continuously by spectrophotometric assays in a coupled lactate dehydrogenase/pyruvate kinase system. Assays are 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-LDH (5U/ml each), 0,85µM AK. The nucleosides, nucleotides, LDH and PK are purchased from Sigma-Aldrich. Reaction is followed in an iEMS Reader MF (Labsystems) microtiter plate reader at 340nm. Assays are performed in duplicate (2 wells per compound and per concentration). Triplicates are available upon request. Km and Vmax are calculated from spectroscopic data using Michaelis-Menten equation.
A confirmation by HPLC analysis of formation of monophosphorylated forms is available upon request.

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

References
1. Wu JZ, Larson G, Walker H, Shim JH, Hong Z. **Phosphorylation of ribavirin and viramidine by adenosine kinase and cytosolic 5'-nucleotidase II: Implications for ribavirin metabolism in erythrocytes**. (2005) *Antimicrob Agents Chemother.* 49(6):2164-71
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Human cytosolic 5'-nucleotidase II (cN-II)

Human, recombinant expressed in E.coli

EC 3.1.3.5

Synonyms: **cytosolic 5'-nucleotidase/phosphotransferase, High Km 5'-nucleotidase (hkm-NT), cytosolic purine 5'-nucleotidase (purine 5'-NT), IMP/GMP-specific 5'-nucleotidase (IMP/GMP-specific 5'-NT)**

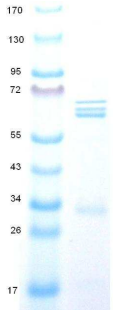
Description

NOVOCIB's human cytosolic IMP/GMP specific 5'-nucleotidase/phosphotransferase II (cN-II) is a recombinant protein of *ca.* 65kDa cloned by RT-PCR amplification of mRNA extracted from human hepatoma cells and expressed in *E.coli*. The sequence of the cloned NT5C2 gene (GenBank accession number P49902) was confirmed by DNA sequencing (100% identity).

Cytosolic 5'-nucleotidase II is one of the seven known mammalian nucleotidases[1] that specifically catalyzes the dephosphorylation of 6-hydroxypurine nucleoside 5'-monophosphates (IMP, dIMP, GMP, dGMP) and regulates cellular pool of IMP and GMP[2, 3]. The enzyme also acts as a phosphotransferase catalyzing the transfer of a phosphate from nucleoside monophosphate to a nucleoside acceptor – preferentially inosine and deoxyinosine. Unlike the other 5'-nucleotidases, cN-II is allosterically regulated by adenine/guanine nucleotides and 2,3-biphosphoglycerate[4].

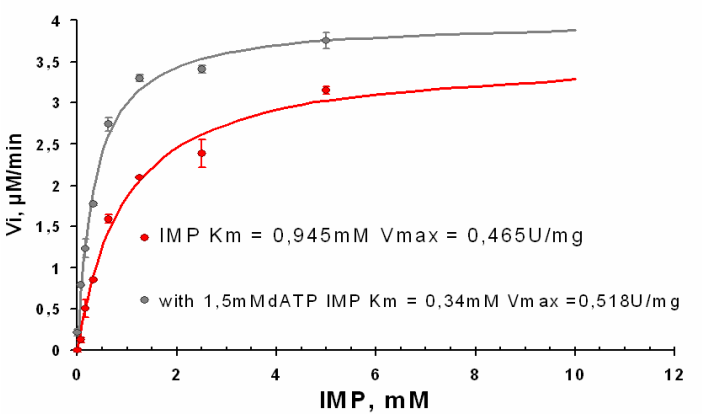
In addition, cytosolic 5'-nucleotidase II phosphorylates anti-viral and anti-tumour nucleoside analogues such as 2'3'-dideoxyinosine, carbovir[5], acyclovir[6] and ribavirin[7].

Storage: –20 ℃ in a solution containing 50 mM Tris-HCl, pH 7.6, 2 mM β-mercaptoethanol, 50% glycerol.



Unit Definition: One unit of 5'nucleotidase converts 1.0 µmole of IMP to inosine per minute at pH 7.6 at 37℃, as measured by a coupled PNP/XO enzyme system in the presence of 20mM MgCl2, 5mM DTT, 500µM KH_2PO_4, and 1,25mM IMP.

Specific Activity:
≥ 0.150 unit/mg protein.
Purity: controlled by 10% AA SDS-PAGE.



5'-nucleotidase assay condition: 5'-nucleotidase activity of cN-II is followed in an irreversible spectrophotometric assay using coupled purine nucleoside phosphorylase - xanthine oxidase system (2,5mU/ml each). Assays were carried out at 37℃, at 50mM Tris-HCl pH7,6; 100mM KCl, 20mM $MgCl_2$, 500µM KH_2PO_4, 5mM DTT, 119nM cN-II and various concentration of IMP. Reaction is followed at 295nm. The IMP is purchased from MP Biochemicals and XO from Sigma-Aldrich. PNP is produced and purified by NovoCIB (ref # E-Nov2).

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.

- ***cN-II phosphorylation assay***
- ***Coupled Nucleoside Kinase – IMPDH II***
- ***Adenosine kinase***
- ***Deoxycytidine kinase (dCK)***
- ***UMP-CMP kinase (CMK)***
- ***dCK nucleoside phosphorylation assay***
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- ***Coupled dCK-CMK nucleoside phosphorylation assays***
- ***Adenosine kinase nucleoside phosphorylation assays***

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Coupled nucleoside kinase - IMPDH II assay

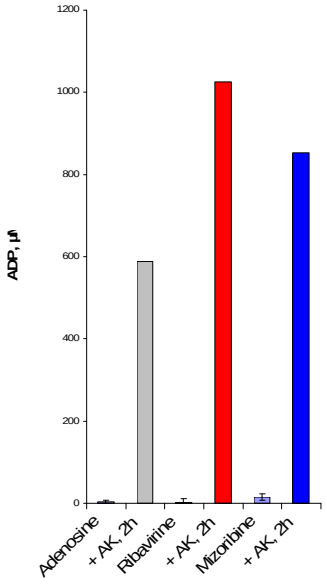
IMPORTANT: Client-specified alterations can be accommodated.

IMP Dehydrogenase (IMPDH, E.C. 1.1.1.205) catalyzes the pivotal step in guanine nucleotide biosynthesis. By converting inosine monophosphate (IMP) to xanthosine monophosphate (XMP), IMPDH controls the guanine nucleotide pool. A number of nucleoside analogues (*e.g.* **ribavirin**, **mizoribine**) are known to inhibit IMPDH after being monophosphorylated. The therapeutic consequences of IMPDH inhibition vary from different analogues - mizoribine is an immunosuppressor and ribavirin is a broad spectrum antiviral. Even if direct relationship between ribavirin antiviral action and IMPDH inhibition by ribavirin monophosphate has not been demonstrated, the depletion of cellular GTP might result in an increased frequency of ribavirin triphosphate incorporation by viral polymerase due to a lower intracellular concentration of its natural competitor.

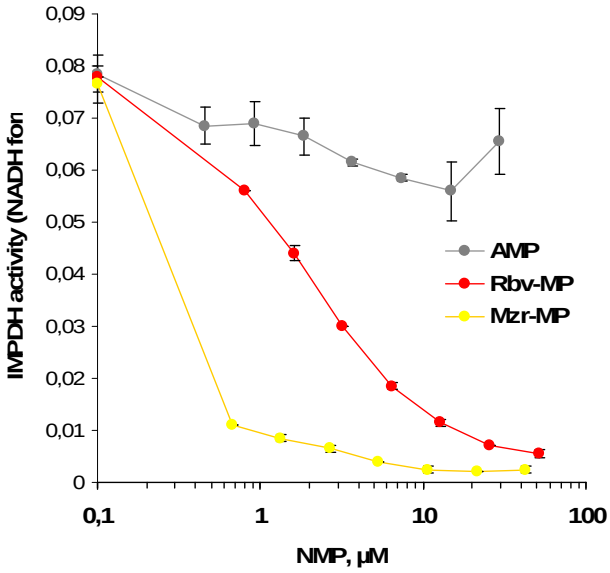
Aim: For rapid evaluation of monophosphate forms of nucleoside analogues as IMPDH inhibitors.

	AK	5'cN-II
Natural substrates	Adenosine Inosine	Deoxyinosine Inosine
Nucleoside analogues substrates	Ribavirin Tubercidin Mizoribine	Dideoxyinosine Ribavirin Acyclovir

Enzymes: The monophosphorylation step of nucleoside analogue is provided by one of the specific human recombinant nucleoside kinases: AK (ref. # E-Nov 5) or cN-II (ref. # E-Nov 6) produced by **NOVOCIB**. Human recombinant IMPDH 2 was cloned from human cells, expressed in *E. coli* and purified by **NOVOCIB** (see sheet # E-Nov 1 for further information). The enzyme purity is controlled by SDS-PAGE, protein concentration is measured by Bradford method (Bio-Rad). A standard operating procedure (SOP) is followed to measure enzymatic activity.



Phosphorylation of nucleoside analogue is provided by specific nucleoside kinase and confirmed by spectrophotometric quantification of formed ADP in LDH-PK coupled reaction.
A confirmation by HPLC analysis of formation of monophosphorylated forms is available upon request.



IMPDH inhibition: Effect of monophosphorylated nucleoside analogues on human recombinant IMPDH II. Enzymatic assays performed in duplicate are carried out at 37°C in 0.1M KH_2PO_4 buffer pH 8.0 in the presence of 2mMDTT, 200µM NAD, 200µM IMP and 0.2 µM IMPDH II and increasing concentration of monophosphorylated nucleoside. Reaction is followed in an iEMS Reader MF (Labsystems) microtiter plate reader at 340nm.

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Related products:

- ***dCK nucleoside phosphorylation assay***
- ***Adenosine kinase nucleoside phosphorylation assays***
- ***cN-II phosphorylation assay***

- ***Deoxycytidine kinase (dCK)***
- ***Adenosine kinase***
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