dCK nucleoside phosphorylation assay

IMPORTANT: Client-specified alterations can be accommodated

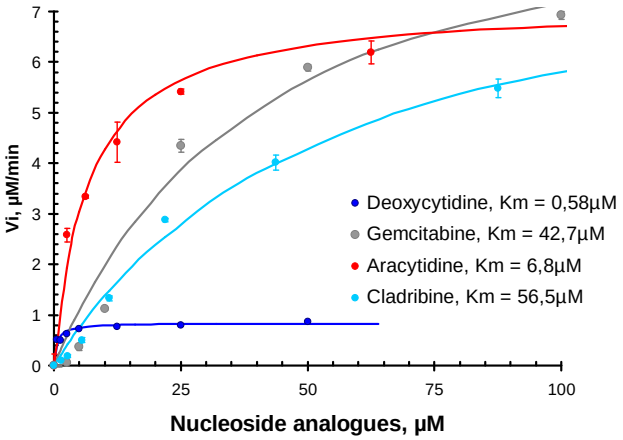
Aim: Characterization of substrate properties (Km and Vmax) of new nucleoside analogues for human deoxycytidine kinase in comparison with the properties of known nucleoside analogues (*e.g.* **aracytidine, gemcitabine, cladribine and lamivudine**).

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: 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.



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.

- ***Deoxycytidine kinase (dCK)***
- ***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] 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