CMK nucleotide monophosphate phosphorylation assay

IMPORTANT: Client-specified alterations can be accommodated.

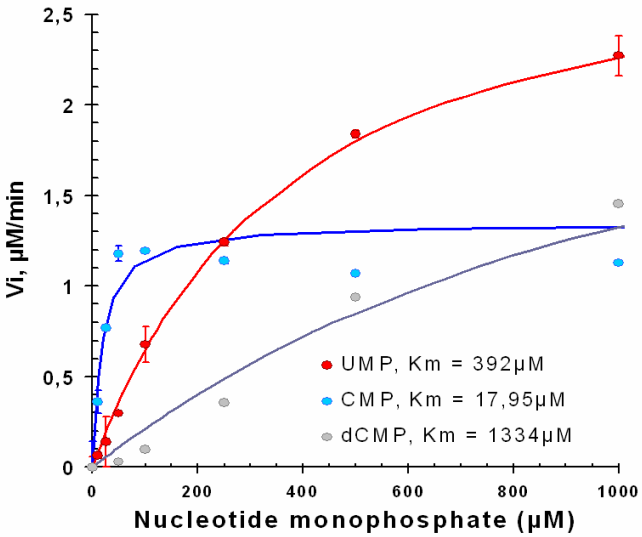
Aim: Characterization of substrate properties (Km and Vmax) of monophosphate forms of new nucleoside analogues for human CMK in comparison with monophosphate forms of natural nucleosides or reference nucleoside analogues.

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.



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.

- ***UMP-CMP kinase (CMK)***
- ***Coupled dCK-CMK nucleoside phosphorylation assays***
- ***Deoxycytidine kinase (dCK)***
- ***Adenosine kinase (AK)***
- ***Cytosolic 5' nucleotidase II (cN-II)***
- ***dCK nucleoside phosphorylation assay***
- ***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

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