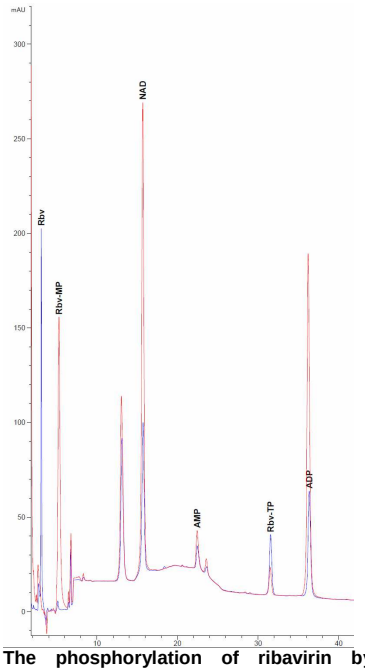
AK nucleoside phosphorylation assay

IMPORTANT: Client-specified alterations can be accommodated.

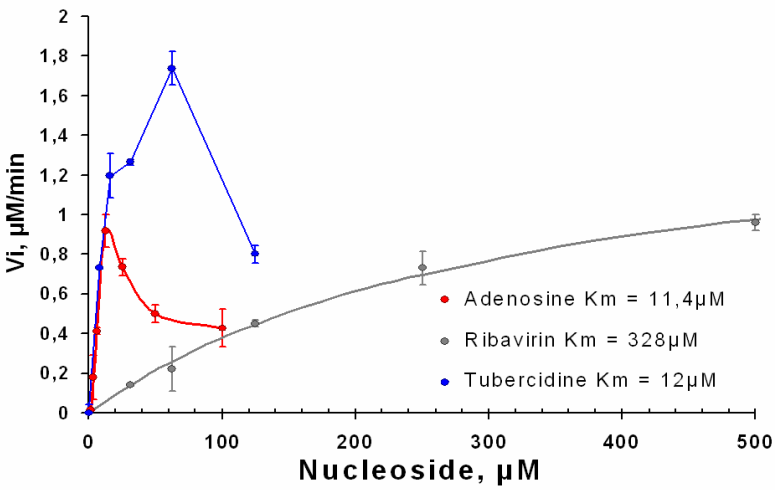
Aim: Characterization of substrate properties (Km and Vmax) of new nucleoside analogues for human adenosine kinase in comparison with properties of known nucleoside analogues (*e.g.* **ribavirine, tubercidine or mizoribine**).

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
2. Yamada, Y.; Goto, H.; Ogasawara, N. **Adenosine kinase from human liver** (1981) *Biochim. Biophys. Act*, 660, 36-43