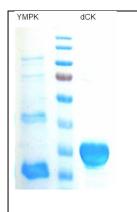
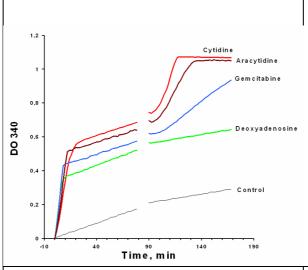


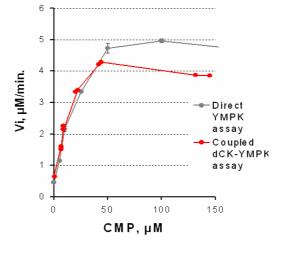
Coupled dCK-CMK nucleoside phosphorylation assays

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 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, 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 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.

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