CMK nucleotide monophosphate phosphorylation assay

**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.

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<thead>
<tr>
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<th>NovoCIB* Published data‡</th>
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<tbody>
<tr>
<td></td>
<td>Km, μM</td>
</tr>
<tr>
<td>CMP</td>
<td>17.9</td>
</tr>
<tr>
<td>UMP</td>
<td>392</td>
</tr>
<tr>
<td>dCMP</td>
<td>1334</td>
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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 MgCl₂, 5mM ATP, 0.1mM NADH, 1mM phosphoenolpyruvate, 1mM DTT, PK 10U/ml, LDH 15U/ml, CMK. Nucleosides, nucleotides, LDH and PK are purchased from Sigma-Aldrich. Reaction is followed in an iEMS Reader MF (Labsystems) microtit 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:**

- **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
