

UMP-CMP kinase (CMK) Human, recombinant expressed in *E.coli* E.C. 2.7.4.14

Synonyms: cytidylate kinase, deoxycytidylate kinase, deoxycytidine monophosphokinase, dCMP kinase, cytidine monophosphate kinase, CMP kinase (CMK, CMPK), uridine monophosphate kinase (UMK, UMPK), uridine monophosphate/cytidine monophosphate kinase, UMP/CMP kinase (UMP/CMPK), CTP:UMP phosphotransferase, ATP:UMP-CMP phosphotransferase, pyrimidine nucleoside monophosphate kinase (YMPK)

Description

NOVO CIB's Human UMP-CMP kinase (CMK) is a recombinant protein of ca. 27kDa (full length 228-aa form¹) cloned by RT-PCR amplification of mRNA extracted from Huh7 cells (human hepatoma) and expressed in *E.coli*.

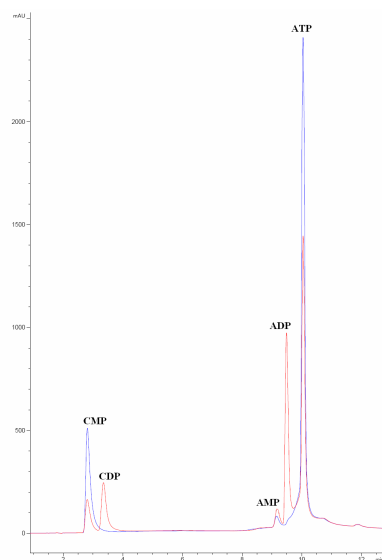
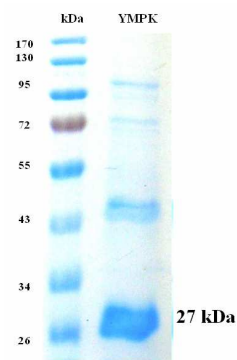
UMP-CMP kinase plays a critical role in supplying cells with nucleotides by catalysing the phosphorylation of CMP, UMP and dCMP to their respective diphosphates. CMK plays also an important role in the activation of cytidine analogues, aracytidine and gemcitabine, a mainstay of leukaemia and lymphoma therapy². CMK has a remarkable ability of to phosphorylate L-nucleotides from their monophosphate to diphosphate forms³ as shown for β -L-2'3'-dideoxy-3'thiacytidine (L-SSdC, 3-TC or lamivudine), an anti-HIV and anti-hepatitis B drug.

Crystal structure of open form of human UMP-CMP kinase has been solved recently⁴. These data, together with the homology model of enzyme in closed state, provides structural basis for understanding the substrate specificity of the enzyme and helps to design new nucleoside analogues of higher phosphorylation efficiency.

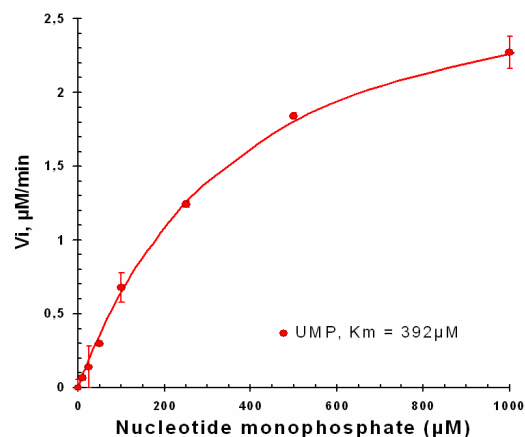
Storage: -20 °C in a solution containing 150mM KCl, 50mM Tris-Hcl, pH7,5, 2mM β -mercaptotethanol, 50% glycerol.

Unit Definition: One unit of UMP-CMP kinase converts 1.0 μ mole of UMP and ATP to UDP and ADP per minute at pH 7.6 at 25°C, using a coupled enzyme system with PK/LDH.

Specific activity: ≥ 2.5 U/mg
Purity: controlled by SDS-PAGE



The enzymatic activity of recombinant UMP-CMP kinase was confirmed by HPLC analysis as illustrated by CDP and ADP formation (red) from CMP and ATP (blue).



Assay condition: Enzymatic activity of UMP-CMP kinase is measured by continuous spectrophotometric assays in a coupled lactate dehydrogenase/pyruvate kinase system. Assays are carried out at 37°C, at 50mM Tris-HCl pH7,6; 50mM KCl, 10mM MgCl₂, 5mM ATP, 0,1mM NADH, 1mM phosphoenolpyruvate, 1mM DTT, PK 10U/ml, LDH 15U/ml, 380nM CMK. Reaction is followed in an iEMS Reader MF (Labsystems, Finland) microtiter plate reader at 340nm.

Related products:

NOVO CIB 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
- 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

¹ Jieh-Yuan Liou, Ginger E. Dutschman, Wing Lam, Zaoli Jiang and Yung-Chi Cheng (March 2002) Characterization of Human UMP/CMP Kinase and Its Phosphorylation of D- and L-Form Deoxycytidine Analogue Monophosphates *Cancer Research* 62, 1624-1631

² Van Rompay AR, Johansson M, and Karlsson A (September 1999) Phosphorylation of Deoxycytidine Analog Monophosphates by UMP-CMP Kinase: Molecular Characterization of the Human Enzyme *Mol Pharmacol* 56 (3), 562-569

³ Claudia Pasti, Sarah Gallois-Montbrun, H el ene Munier-Lehmann, Michel Veron, Anne-Marie Gilles and Dominique Deville-Bonne (Mar2003) Reaction of human UMP-CMP kinase with natural and analog substrates. *European Journal of Biochemistry*, 270 (8), 1784 - 1790

⁴ Segura-Pe a D, Sekulic N, Ort S, Konrad M, Lavie A. (2004) Substrate-induced conformational changes in human UMP/CMP kinase. *J Biol Chem*. 279(32):33882-33889