

Human cytosolic 5'-nucleotidase II (cN-II)

Human, recombinant expressed in E.coli

EC 3.1.3.5

Synonyms: cytosolic 5'-nucleotidase/phosphotransferase, High Km 5'-nucleotidase (hkm-NT), cytosolic purine 5'-nucleotidase (purine 5'-NT), IMP/GMP-specific 5'-nucleotidase (IMP/GMP-specific 5'-NT)

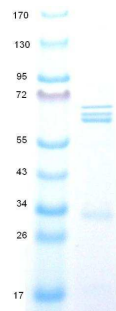
Description

NOVO CIB's human cytosolic IMP/GMP specific 5'-nucleotidase/phosphotransferase II (cN-II) is a recombinant protein of ca. 65kDa cloned by RT-PCR amplification of mRNA extracted from human hepatoma cells and expressed in *E.coli*. The sequence of the cloned NT5C2 gene (GenBank accession number P49902) was confirmed by DNA sequencing (100% identity).

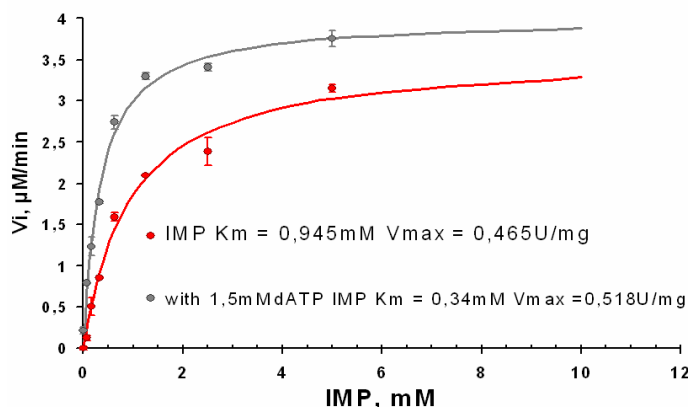
Cytosolic 5'-nucleotidase II is one of the seven known mammalian nucleotidases¹ that specifically catalyzes the dephosphorylation of 6-hydroxypurine nucleoside 5'-monophosphates (IMP, dIMP, GMP, dGMP) and regulates cellular pool of IMP and GMP^{2,3}. The enzyme also acts as a phosphotransferase catalyzing the transfer of a phosphate from nucleoside monophosphate to a nucleoside acceptor – preferentially inosine and deoxyinosine. Unlike the other 5'-nucleotidases, cN-II is allosterically regulated by adenine/guanine nucleotides and 2,3-bisphosphoglycerate⁴.

In addition, cytosolic 5'-nucleotidase II phosphorylates anti-viral and anti-tumour nucleoside analogues such as 2'3'-dideoxyinosine, carbovir⁵, acyclovir⁶ and ribavirin⁷.

Storage: -20 °C in a solution containing 50 mM Tris-HCl, pH 7.6, 2 mM β-mercaptoethanol, 50% glycerol.



Unit Definition: One unit of 5'-nucleotidase converts 1.0 μmole of IMP to inosine per minute at pH 7.6 at 37°C, as measured by a coupled PNP/XDH enzyme system in the presence of 20mM MgCl₂, 5mM DTT, 500μM KH₂PO₄, and 1,25mM IMP.



5'-nucleotidase assay condition: 5'-nucleotidase activity of cN-II is followed in an irreversible spectrophotometric assay using coupled purine nucleoside phosphorylase - xanthine dehydrogenase system (2,5mU/ml each). Assays were carried out at 37°C, at 50mM Tris-HCl pH7,6; 100mM KCl, 20mM MgCl₂, 500μM KH₂PO₄, 5mM DTT, 119nM cN-II and various concentration of IMP. Reaction is followed at 295nm. The IMP is purchased from MP Biochemicals. PNP and XDH enzymes are produced and purified by NovoCIB.

Specific Activity:

≥ 0.150 unit/mg protein.

Purity: controlled by 10% AA SDS-PAGE.

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.

- **cN-II phosphorylation assay**
- **Coupled Nucleoside Kinase – IMPDH II**
- **Adenosine kinase**
- **Deoxycytidine kinase (dCK)**
- **UMP-CMP kinase (CMK)**
- **dCK nucleoside phosphorylation assay**
- **CMK nucleotide monophosphate phosphorylation assay**
- **Coupled dCK-CMK nucleoside phosphorylation assays**
- **Adenosine kinase nucleoside phosphorylation assays**

¹ Bianchi V, Spychala J. **Mammalian 5'-nucleotidases** (2003) *J. Biol Chem* 278(47): 46195-46198

² Allegrini S, Pesi R, Tozzi MG, Fiol CJ, Johnson RB, Eriksson S. **Bovine cytosolic IMP/GMP-specific 5'-nucleotidase: cloning and expression of active enzyme in Escherichia coli.** (1997) *Biochem J.* 328:483-7.

³ Ipata PL, Tozzi MG **Recent advances in structure and function of cytosolic IMP-GMP specific 5'-nucleotidase II (cN-II)** (2006) *Purinergic Signal.* 2(4):669-75

⁴ Spychala J, Madrid-Marina V, Fox IH **High Km Soluble 5'-nucleotidase from human placenta** (1988) *J Biol Chem* 263(35): 18759-18765

⁵ Johnson MA, Fridland A. **Phosphorylation of 2',3'-dideoxyinosine by cytosolic 5'-nucleotidase of human lymphoid cells.** (1989) *Mol Pharmacol.* 36(2):291-5

⁶ Keller PM, McKee SA, Fyfe JA. **Cytoplasmic 5'-nucleotidase catalyzes acyclovir phosphorylation** (1985) *J Biol Chem.* 260(15):8664-7

⁷ 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