

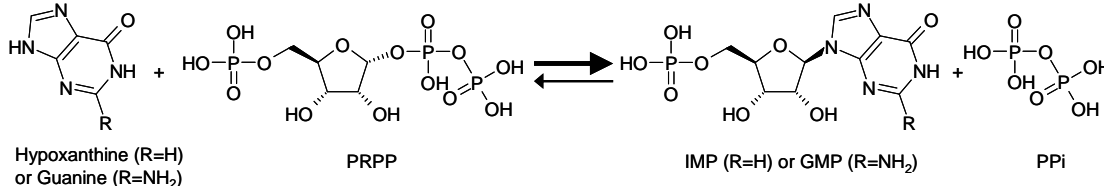
Human HGPRT Human, recombinant expressed in *E.coli* EC 2.4.2.8

Synonyms: Hypoxanthine phosphoribosyltransferase, HGPRTase, HPRT

Description

NOVO CIB's human hypoxanthine-guanine phosphoribosyl transferase (HGPRT) is a recombinant protein of ca.25kDa cloned by RT-PCR amplification of mRNA extracted from human hepatoma cells and expressed in *E.coli*. The sequence of the cloned HGPRT (GenBank accession number P00492) was confirmed by DNA sequencing (100% identity).

Hypoxanthine-guanine phosphoribosyltransferase (HGPRTase; EC 2.4.2.8) is a purine salvage enzyme that catalyzes the reversible transfer of the 5-phosphoribosyl group between α -D-5-phosphoribosyl-1-pyrophosphate (PRPP) and a purine base (hypoxanthine or guanine) to form a purine nucleotide IMP or GMP. The reaction is reversible but forward reaction (nucleotide formation) is heavily favoured.



HGPRTase has been extensively investigated because defects within the human enzyme are associated with genetically inherited gouty arthritis and Lesch-Nyhan syndrome¹.



Form: lyophilized form. Stored at -20°C.

Unit Definition (forward reaction):

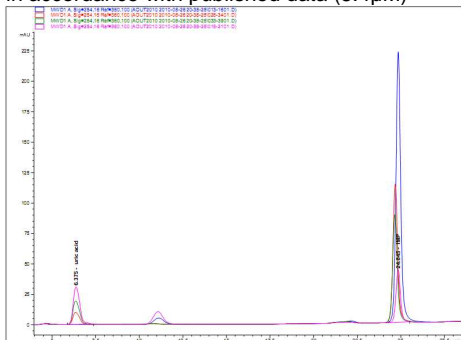
One unit of hypoxanthine-guanine phosphoribosyl transferase converts 1 μ mole of Hypoxanthine to IMP per minute at pH 7.5 at 37 °C, as measured by a coupled bacterial IMPDH (NovoCIB) enzyme system.

Specific Activity: ≥ 0.8 unit/mg protein (forward reaction).

Purity: controlled by 10%AA SDS-PAGE.

Assay conditions (reverse reaction): Enzymatic activity of HGPRT is measured by spectrophotometric assays in a coupled xanthine dehydrogenase (XDH, NovoCIB) system.

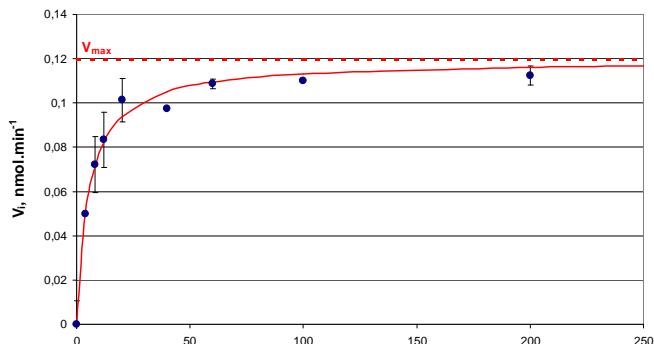
Assays were carried out at 37°C, in 50mM Tris-HCl pH 7.5; 12mM Mg Acetate, 2mM NAD, 1mM PPi. Reaction started by adding IMP at various concentrations. NADH formation was measured in an iEMS Reader MF (LabSystems, Finland) microtiter plate reader at 340nm. Lineweaver-Burk plotting gave a $K_M = 5.448 \mu\text{M}$, which is in accordance with published data ($5.4 \mu\text{M}$)⁽¹⁾



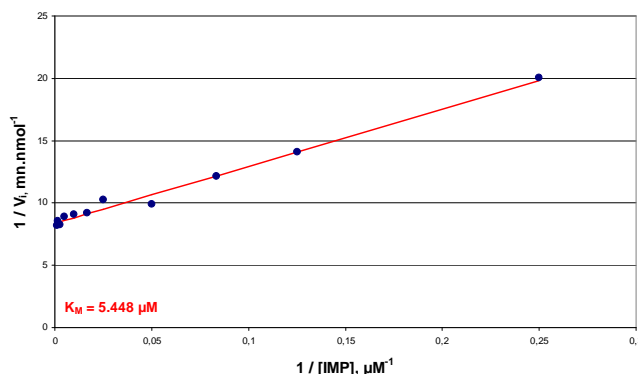
The enzymatic activity (reverse reaction) of human recombinant HGPRT was confirmed by ion-pair HPLC analysis (Agilent 1100 series, Zorbax C18plus) as shown by time-dependent decrease in IMP (blue-0min; red- 30min; green-3h; pink-24h) incubation at 37°C. To avoid forward reaction, hypoxanthine formed due to IMP pyrophosphorolysis was converted to uric acid due to added XDH.

Since the hydrolysis of nucleotides analogues of inosine or guanosine by HGPRT may limit their efficiency, **NOVO CIB** has developed an HGPRT enzymatic assay which consists in treating monophosphate Nucleoside Analogues by HGPRT and evaluating by HPLC their resistance to pyrophosphorolysis by human recombinant HGPRT. The reverse enzymatic reaction (IMP pyrophosphorolysis) is measured by a coupled XDH (**NOVO CIB**) enzyme system.

HGPRT initial velocity at various IMP concentrations



Lineweaver-Burk plots for IMP as variable substrate



¹ Balendiran GK, Molina JA, Xu Y, Torres-Martinez J, Stevens R, Focia PJ, Eakin AE, Sacchettini JC, Craig SP 3rd. (1999) Ternary complex structure of human HGPRTase, PRPP, Mg2+, and the inhibitor HPP reveals the involvement of the flexible loop in substrate binding *Protein Science*, 8(5),1023–1031.