Human HGPRT

Human, recombinant expressed in E.coli

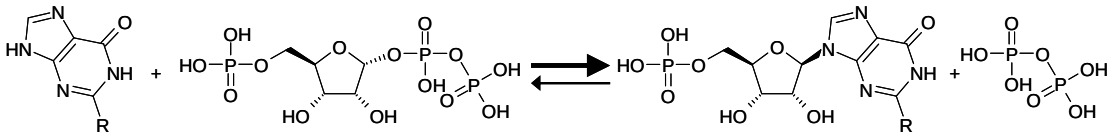
EC 2.4.2.8

Synonyms: Hypoxanthine phosphoribosyltransferase, HGPRTase, HPRT

Description

NOVOCIB's human hypoxanthine-guanine phosphoribosyl transferase (HGPRT) is a recombinant protein of *ca.25*kDa 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.



Hypoxanthine (R=H) or Guanine (R=NH_2) + PRPP ⇌ IMP (R=H) or GMP (R=NH_2) + PPi

HGPRTase has been extensively investigated because defects within the human enzyme are associated with genetically inherited gouty arthritis and Lesch–Nyhan syndrome[1].

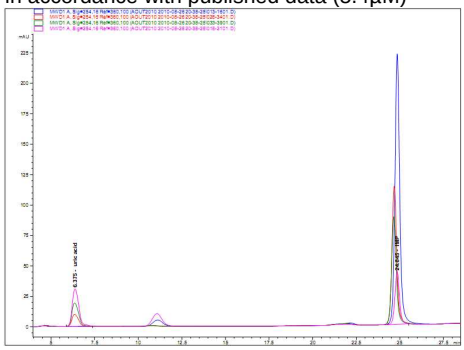
Form: lyophilized form. Stored at -20℃.

Unit Definition (forward reaction): One unit of hypoxanthine-guanine phosphoribosyl transferase converts 1 µmole of Hypoxanthine to IMP per minute at pH 7.5 at 37 ℃, as measured by a coupled bacterial IMPDH (NovoCIB) enzyme system.

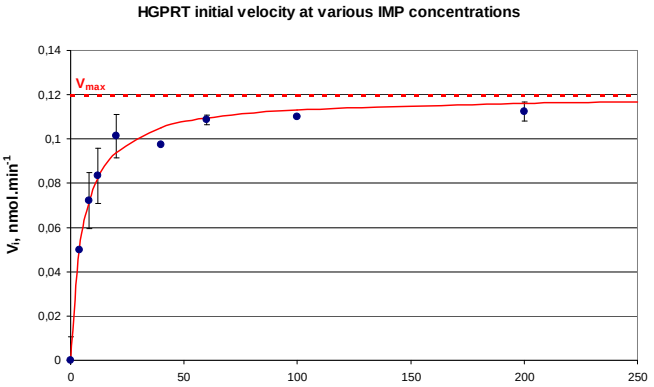
Specific Activity: ≥ 0.8 unit/mg protein (forward reaction).

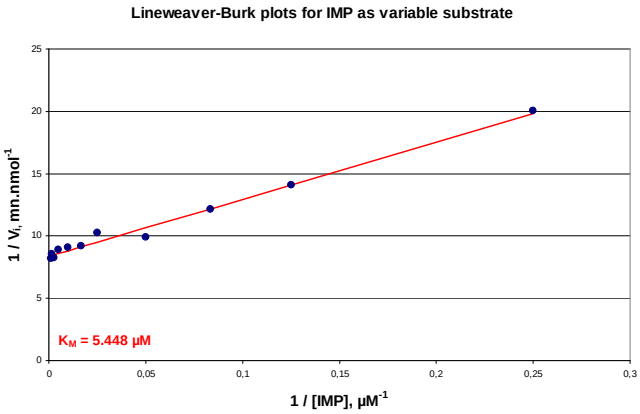
Purity: controlled by 10%AA SDS-PAGE.

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℃, in 50mM Tris-HCl p H 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 KM = 5.448µM, which is in accordance with published data (5.4µM)[(1)]



Since the hydrolysis of nucleotides analogues of inosine or guanosine by HGPRT may limit their efficiency, **NOVOCIB** 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 (**NOVOCIB**) enzyme system.





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℃. To avoid forward reaction, hypoxanthine forme d due to IMP pyrophosphorolysis was converted to uric acid due to added XDH.

[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, Mg21, and the inhibitor HPP reveals the involvement of the flexible loop in substrate binding** *Protein Science*, 8(5),1023–1031.

NovoCIB 115 avenue Lacassagne
69003 Lyon, France
Tel / Fax +33 (0)478536395
contact@novocib.com www.novocib.com