HGPRT One-Step Assay Kit
Hypoxanthine-guanine phosphoribosyl transferase Assay

Hypoxanthine-guanine phosphoribosyltransferase (HGPRT, EC 2.4.2.8) has been extensively investigated because defects within the human enzyme are associated with genetically inherited gouty arthritis and Lesch–Nyhan syndrome[1].

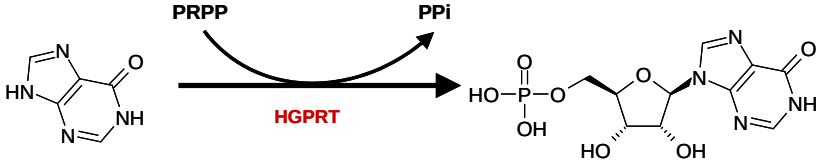
Active HGPRT is also involved in the activation of purine analogues pro-drugs, such as 6-methylmercaptopurine or azathioprine[2].

NOVOCIB's HGPRT Assay Kit is designed to measure the activity of HGPRT (hypoxanthine-guanine phosphoribosyl transferase) *in vitro*.

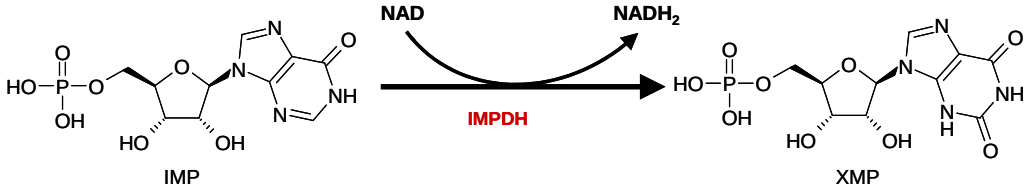
This very easy **one-step Assay** is based on a coupled reaction involving a highly active IMPDH (Inosine Monophosphate Dehydrogenase, bacterial recombinant) which directly measures the IMP formed by HGPRT present in the sample.

Principle
NOVOCIB's HGPRT Assay Kit involves the coupling of the following reactions:

(1) In the presence of Hypoxanthine, HGPRT in the sample catalyses the transfer of the 5-phosphoribosyl group of α-D-5-phosphoribosyl-1-pyrophosphate (PRPP) to form Inosine Monophosphate (IMP) and release a pyrophosphate:



(2) IMP is immediately oxidized to Xanthine Monophosphate (XMP) by a highly active IMPDH in the presence of NAD.



In its HGPRT one-step Assay, **NOVOCIB** uses a bacterial recombinant IMPDH which shows a strong specific activity. When in excess, this IMPDH permits a direct measurement of HGPRT activity through $NADH_2$ formation. This coupling system also presents the advantage of enhancing the measurement of HGPRT activity by converting instantaneously the IMP formed, thus preventing from reversible HGPRT reaction.

$NADH_2$ formation is followed spectrophotometrically at 340 nm. The rate of $NADH_2$ formation is directly proportional to the rate of phosphoribosylation of hypoxanthine, catalyzed by HGPRT in the sample.

NOVOCIB's HGPRT one-step assay was validated on Red Blood Cell lysate.

[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.
[2] Estlin EJ, Lowis SP, Hall AG. (2000) **Optimizing antimetabolite-based chemotherapy for the treatment of childhood acute lymphoblastic leukemia** *Br. J. Haematol.*, 110(1),29-40.

Assay with Red Blood Cells (RBC) lysate:
Figure 1 shows HGPRT activity of RBC lysate measured spectrophotometrically at 340nm using **NOVOCIB**'s HGPRT Assay.

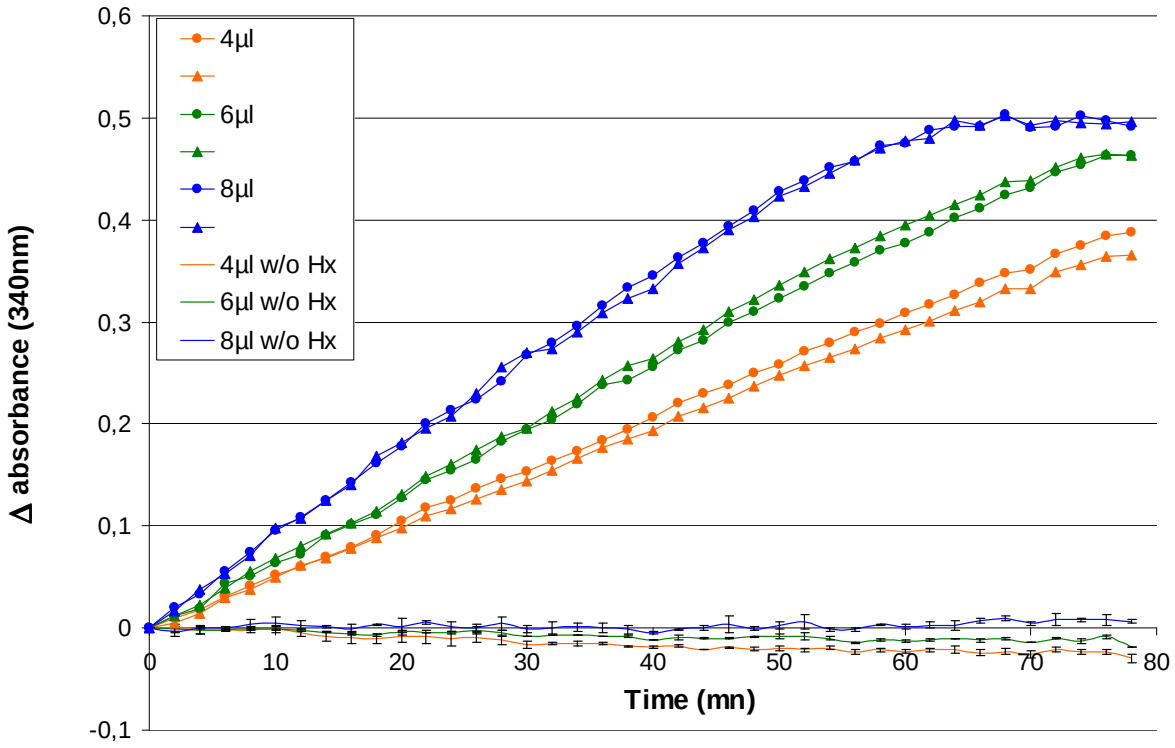


Figure 1: HGPRT activity of RBC lysate measured using a coupled one-step IMPDH enzymatic system.
Assays were done in duplicate for 4µl, 6µl and 8µl of RBC lysate in 200µl well.Negative controls are indicated by curves with no marker points (mean +/- SD)

RBC lysate was prepared from a 75µl-pellet of RBCs previously washed in PBS. After resuspension in 300µl (4v) of ice cold water and 10 mn of centrifugation at 14,000 g, 4℃, the clear red RBC lysate was removed and stored on ice for performing the HGPRT assays.
4µl, 6µl and 8µl of RBC lysate were used to measure HGPRT activity in 200µl of reaction buffer. Assays were performed in duplicate on a 96-well microplate. NADH formation was measured in an iEMS Reader MF (Labsystems, Finland) microtiter plate reader at 340nm.
After 10 mn of incubation at 37℃, reaction was started by adding 10µl of 20mM Hypoxanthine solution (final concentration: 1mM).
Blank with no RBC lysate was run in duplicate (not shown). Negative controls consisting in wells containing 4, 6 and 8µl of RBC lysate where no Hypoxanthine was added were also run in duplicate (orange, green and blue marker-less lines).

Kit Content
Reaction buffer x10
Reagent Mix (PRPP, NAD…) w/o Hypoxanthine
Hypoxanthine solution
IMPDH enzyme, lyophilized *

Positive control can be run using **NOVOCIB**'s HGPRT enzyme (lyophilized) ** (upon request)

* Recombinant IMPDH from *Staphylococcus aureus*, expressed in *E. coli* (Ref. #E-Nov7)
** Human recombinant HGPRT, expressed in *E. coli* (Ref. #E-Nov9)