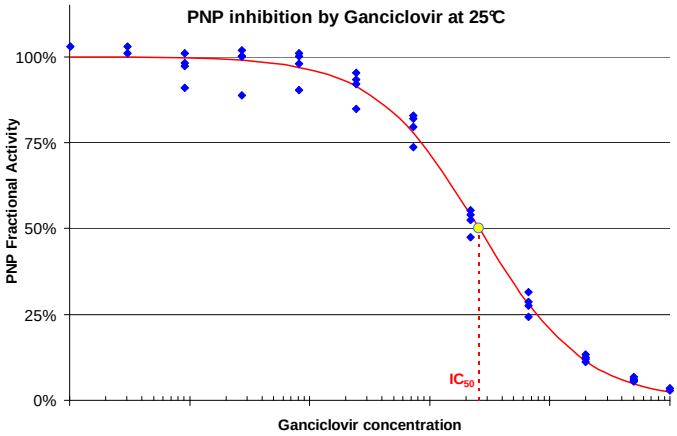
PNP Inhibition - *In vitro* Screening Assay

IMPORTANT: Client-specified alterations can be accommodated.

Aim: To screen compounds for their abilities to inhibit human PNP *in vitro*.

Description of the *In vitro* screening assay

PNP enzyme used in the assay is a human recombinant PNP, cloned by **NOVOCIB** from human cells, expressed in *E. coli*, and produced and purified in **NOVOCIB**'s laboratory. **(see sheet # E-Nov 2 for further information)** PNP purification is controlled before every assay by SDS-PAGE. Protein concentration is measured by Bradford method. PNP specific activity is then determined - 1 unit of PNP catalyzes the cleavage of 1 µmole of inosine per minute at pH 8.0 at 25 ℃



Procedure
NOVOCIB has developed a spectrophotometric procedure to directly follow the PNP phosphorolytic reaction on inosine (IR).
The assays are performed at 25℃ or 37℃ in 200µl o f reaction buffer on 96-well microplate. Pipetting is done by a Multiprobe® II Robotic Liquid Handling System (Packard BioScience). Ganciclovir is used as positive control for PNP inhibition.
Replicate assays: One point is defined as a well per compound and per concentration tested. *In vitro* PNP Inhibition Screening Assays are usually performed in duplicate (2 wells per compound and per concentration). Both negative and positive controls are done in duplicate. Triplicates are available upon request.
Every assay, from one to 90 points, is done with one negative control, *i.e.* without inhibitor, and two positive controls containing Ganciclovir as a PNP inhibitor. Controls are done in duplicate. If an additional microplate is needed, it includes the three controls (in duplicate, *i.e.* 6 wells).

(Optional) Confirmation by HPLC:
For every positive assay, an HPLC (Agilent 1100 series) control of PNP inhibition can be performed by measuring inosine (IR) and Hypoxanthine (Hx) concentrations in the assay and in comparison with negative and positive controls.

NOVOCIB has cloned and purified a human recombinant Purine Nucleoside Phosphorylase (PNP) and has developed a range of related PRECICE® services.

PNP Services

- **PNP Inhibition - *In Vitro* Screening Assay**
- **PNP Cleavage activity - Nucleoside Resistance Assay**
  (PNP enzyme can also be used for transribosylation)

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