

## PNP Cleaving activity - Nucleoside Resistance Assay

**IMPORTANT:** Client-specified alterations can be accommodated.

**Aim:** To evaluate the resistance of Nucleoside Analogues to cleavage by human PNP.

### Nucleoside Analogues deactivation by PNP

*In vivo*, phosphorolysis is highly favoured over purine nucleoside synthesis and is coupled with two additional enzymatic reactions: oxidation of the liberated purine base by xanthine oxidase (XO) and its phosphoribosylation by hypoxanthine-guanine phosphoribosyltransferase (HGPRT)<sup>1</sup>. Thus, PNP plays a key role in the salvage pathway of the purine metabolism, enabling the cell to utilize purine bases recovered from metabolized purine ribo- and deoxyribonucleosides to synthesize purine nucleotides.

This phosphorolysis reaction of purine nucleosides by PNP has a direct impact on the therapeutic efficacy of Nucleoside Analogues. Antitumour or antiviral nucleoside analogues are likely to be cleaved by PNP before being phosphorylated by the cell nucleoside kinases and converted to the active nucleotide form. For instance, 2',3'-dideoxyguanosine (ddG)<sup>2</sup>, 9-β-D-arabinofuranosyl guanine (AraG)<sup>3</sup> as well as one of its produg, Nelarabine (Arranon®, GSK)<sup>4</sup>, which is intracellularly converted to AraG by Adenosine deaminase (ADA), are PNP resistant nucleoside analogues, whereas 2',3'-dideoxyinosine (ddI)<sup>5</sup> is easily cleaved *in vivo* by PNP.

Since acyclonucleoside analogues are particularly resistant to cleavage by PNP though phosphorylated by viral thymidine kinases (TK), they are generally considered as excellent candidates as antiviral agents (e.g. aciclovir, ganciclovir)<sup>6</sup>.

*Note that Ganciclovir is not only PNP resistant, but is also a PNP inhibitor.*

NOVO CIB's has developed a PNP enzymatic assay which consists in evaluating the PNP phosphorolysis activity on Nucleosides Analogues in comparison with natural purine nucleoside substrates.

### Description of the Nucleoside Resistance assay

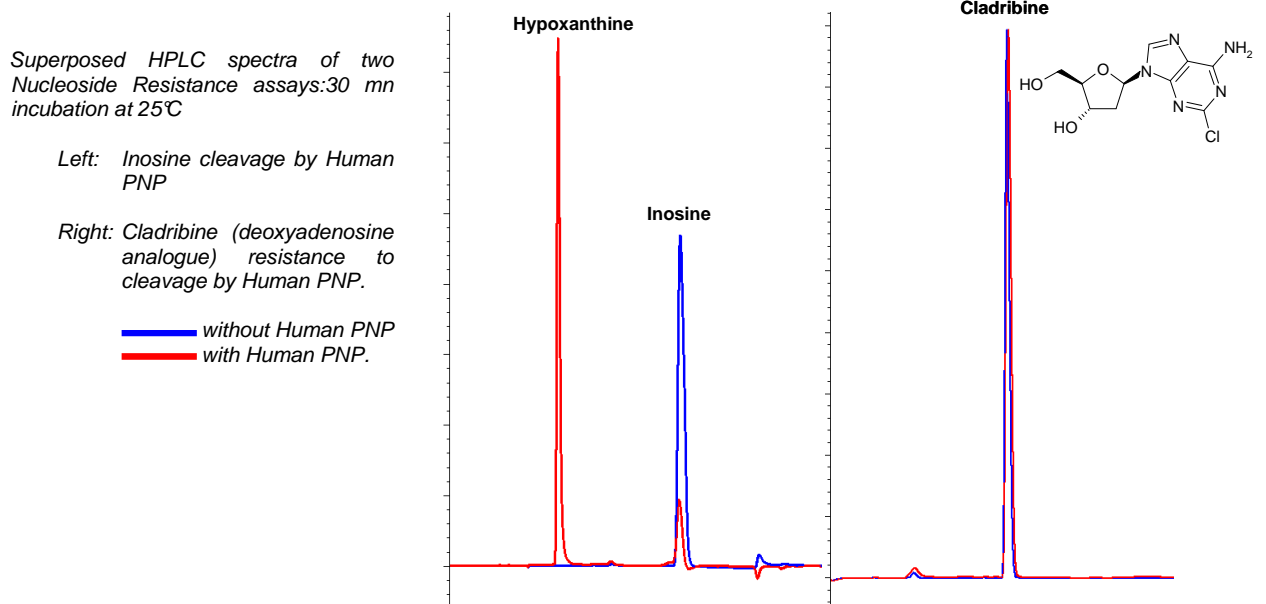
The assays are performed at 25°C or 37°C in 200µl of reaction buffer (lower volumes are available if compound saving is a constraint) on 96-well microplate. Pipetting is done by a Multiprobe® II Robotic Liquid Handling System (Packard BioScience).

Inosine is used as a positive control and adenosine as a negative control.

*Note that if inosine is a natural substrate of PNP and is consequently actively cleaved by a wide range of PNPs, adenosine resists to Human PNP phosphorolytic activity but is easily cleaved by other PNPs, such as E.coli PNP for instance.*

**This is why we consider as a decisive advantage to evaluate the cleavage resistance of Nucleoside Analogue using Human PNP.**

Identification and quantification of the purine nucleosides and the related purine bases produced by Human PNP cleaving activity are performed by HPLC.



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3. L. C. Gravatt *et al.* (1993): Efficacy and toxicity of 9-β-D-arabinofuranosylguanine (araG) as an agent to purge malignant T-cells from murine bone marrow: application to an *in vivo* T-cell leukemia model, *Leukemia* 7(8), 1261-1267
4. C. U. Lambe *et al.* (1995): 6-Amino-6-methoxypurine arabinoside: an agent for T-cell malignancies, *Cancer Res.* 55(15), 3352-3356
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6. D. Shugar (1999): Viral and host-cell protein kinases: enticing antiviral targets, and relevance of nucleoside, and viral thymidine, kinases, *Pharmacol. Ther.* 82(2-3), 315-335