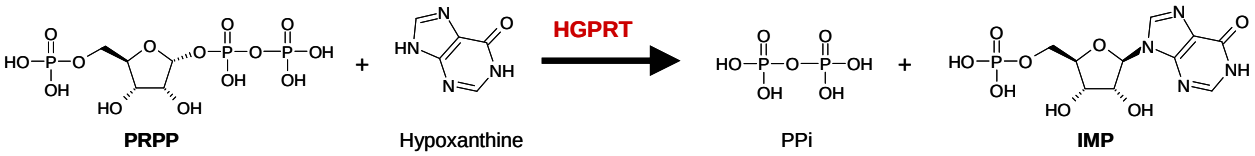
PRPP Assay Kit

For a one-step enzymatic measurement of α-D-5-phosphoribosyl-1-pyrophosphate (PRPP)

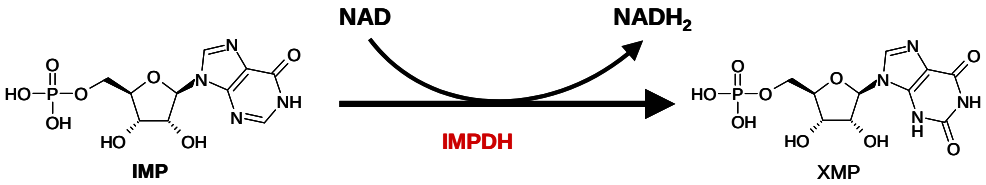
NOVOCIB's **PRPP Assay Kit** is designed to measure PRPP (α-D-5-phosphoribosyl-1-pyrophosphate) content in samples. This enzymatic assay is based on a coupled reaction involving Hypoxanthine-guanine phosphoribosyltransferase (HGPRT) and Inosine Monophosphate Dehydrogenase (IMPDH).
PRPP is highly unstable, which renders the preparation of accurately weighted standards difficult. **NOVOCIB**'s PRPP Assay Kit has the advantage of measuring PRPP concentration in the sample by absorbance through a stoichiometric $NADH_2$ formation, thus allowing a direct and absolute measurement of PRPP content with no need of any calibration curve.

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

(1) In the presence of Hypoxanthine, the 5-phosphoribosyl group of PRPP is first transferred by Hypoxanthine-guanine phosphoribosyltransferase (HGPRT) to form Inosine Monophosphate (IMP) and release a pyrophosphate:



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



The $NADH_2$ formed is equivalent to the amount of PRPP present in the assay. $NADH_2$ formation is directly monitored spectrophotometrically at 340 nm.

Assay with a range of PRPP standards
Figure 1 shows the correlation between PRPP concentrations of standards ranging from 2 to 400µM in 200µl-well assays and the PRPP concentrations as measured spectrophotmetrically at 340nm by applying a 0.625cm ligh-path (corresponding to 200µl/well in a standard 96-well microplate).

Kit Content
Reaction buffer x10
Reagents: NAD, Hypoxanthine
IMPDH enzyme, lyophilized *
HGPRT enzyme, lyophilized **

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

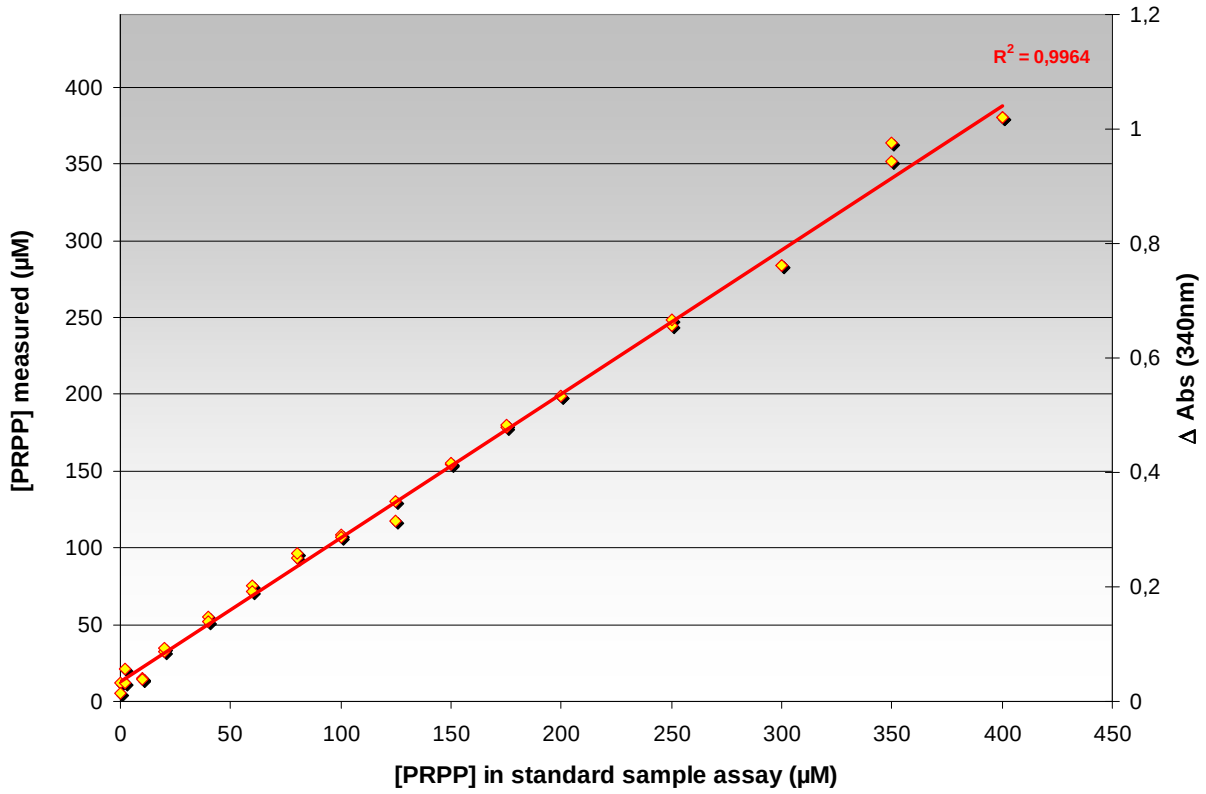


Figure 1: Correlation between PRPP concentration of the standards and PRPP concentration measured with NOVOCIB's PRPP Assay Kit.

Assays were done in duplicate using a range of freshly prepared PRPP (Sigma-Aldrich, P8296). The concentration of the standards indicated on the graphic takes into account the 75% purity indicated by the supplier.

Due to PRPP instability, standards may be difficult to prepare. This correlation curve shows that a direct measurement of PRPP content can be done without any standard but directly through the absorbance of the $NADH_2$ formed in the assay after 35 minutes of reaction.