PRECICE® PRPP Assay Kit

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

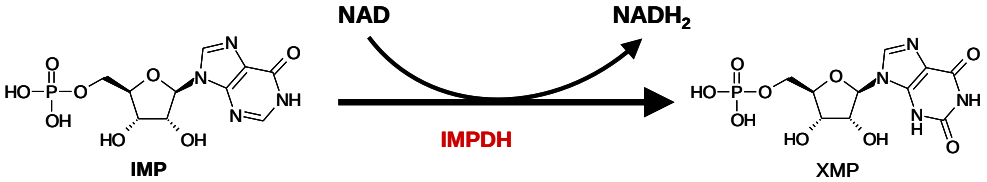
I. Introduction

PRECICE® 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 PRECICE® PRPP Assay Kit offers 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.

The principle of the assay is based on the coupling of the following enzymatic reactions

(1) In the presence of **Hypoxanthine** (Hx), 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.

Warning: *PRECICE® PRPP Assay Kit allows PRPP quanitification within the range of 5 to 400µM and does not allow to mesure PRPP in mammalian cells where its concentration varies from 0.1-1µM.*
The following instructions are given to measure the concentration of PRPP for R&D use only. NovoCIB does not guarantee the use of its PRECICE® PRPP Assay Kit or of one or several of its component, in other conditions and/or for other purpose than those described in this user manual.

II. Equipments required

1) Plate agitator
2) Plate reader fitted with a filter 340nm (ex. Labsystems iEMS Reader MF (Thermo), Epoch (BioTec); PerkinElmer.

III. Kit Contents for 24 analysis (8 samples in triplicate):

Once dissolved, the reagents provided in the kit are not stable and should be used immediately..
A standard PRECICE® PRPP Assay Kit contains:

- one tube **"Cofactor 1"** (DTT);
- one tube **"Cofactor 2"** (NAD);
- one tube **" IMPDH enzyme"** (>500mU);
- one tube **" HGPRT enzyme"** (>500mU);
- one 15-ml tube **"Reaction buffer BLANK"** (10mL, blue cap, BD®);
- one 15-ml tube **"Reaction buffer Hx+"** (contains 5µmol of hypoxanthine, orange cap Corning ®);
- one transparent 96-well plate (round-bottom 96-well plate Corning, Costar®, ref. 3797)

Not provided:
Deionized water
Positive control: PRPP (α–D-5-phosphoribosyl-1-pyrophosphate, available at Sigma-Aldrich, ref. P8296)

IV. Recommendations for PRPP extraction

- ✓ *Several protocols of PRPP extraction have been described:*
  1. *chemical extraction where proteins are precipitated with perchloric acid (PCA) or trichloroacetic acid (TCA).*
  2. *Extraction by boiling where cells or tissue is heated for 85°C.*

- ✓ *Since both PCA and TCA were found to inhibit enzymes used in PRECICE ® PRPP Assay, we strongly recommend to perform boiling extraction rather than chemical extraction.*

- ✓ *The addition of EDTA to extraction buffer should be avoided since HGPRT enzyme requires the presence of divalent cations.*

- ✓ *To be sure that the procedure of PRPP extraction did work, we recommend to use "spiked" samples (samples where known concentration of PRPP was added to the samples) as positive controls.* ***Important:*** *PRPP is highly unstable once dissolved. We recommend to prepare the tubes with inducated mg of PRPP, store them as a powder at -20°C and dissolve it at very last moment.*

For additional information contact us at contact@novocib.com)

V. Preparation of reaction mixtures (48 assays of 200µL, 24 with and 24 without Hx)

1. Add 500µL of deionized water to the tube with **"Cofactor 1"** to prepare 0.1M DTT. Agitate until complete dissolution of the powder.

2. Transfer dissolved **"Cofactor 1"** to the tube with **"IMPDH enzyme"**. Agitate until complete dissolution of the powder.

3. Add 200µL of deionized water to the tube with **"Cofactor 2"**. Agitate until complete dissolution of the powder.

4. Add 200µL of deionized water to the tube with **"HGPRT enzyme"**. Agitate until complete dissolution of the powder.

4. Quantitaviely transfer the content of 4 tubes with **"Cofactor 1", "Cofactor 2", "HGPRT enzyme"** and **"IMPDH enzyme"** to a blue 15-ml tube "**Reaction mixture BLANK"**

To do so:

-pipet 1ml of buffer from **"Reaction mixture 1x"** to each tube and mix them by inverting or pipeting up and down until the powder dissolved.

- transfer the content of two tubes back into a vial **"Reaction mixture 1x"** by pipeting.

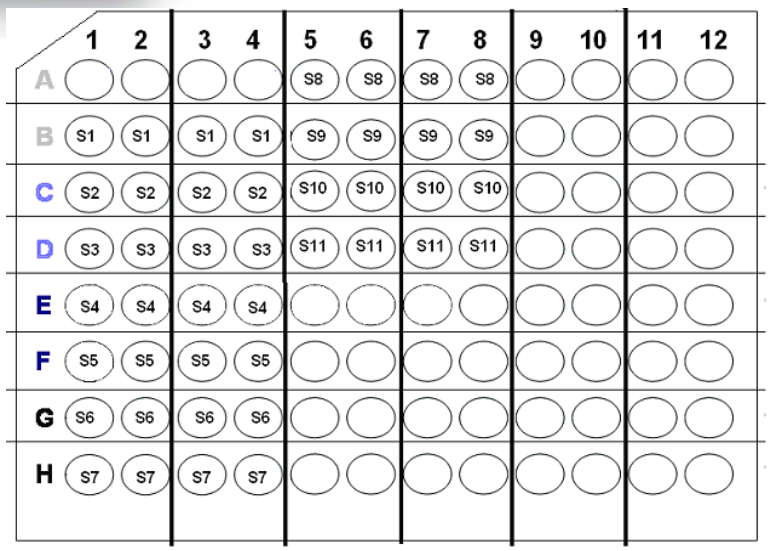
- repeat to be sure that all reagent and enzymes of the small tubes and vial are recovered. mix by gently inverting until complete dissolution. Avoid bubbles.

4. Transfer 5ml of complete **"Reaction mixture 1x"** containing enzymes and cofactors to orange 15ml tube pre-filled with 100µL of 50mM hypoxanthine solution**.**

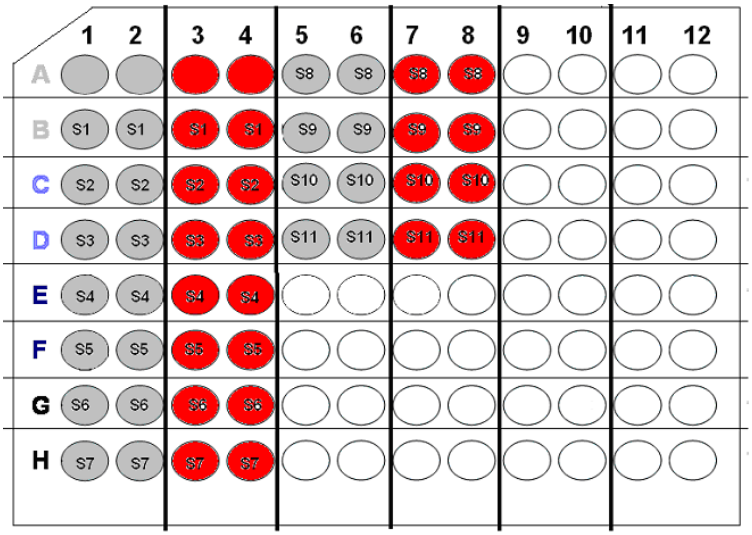
You have prepared: 5ml of **Reaction mixture BLANK**
5ml of **Reaction mixture Hx+**

VI. Microplate preparation for analysis of 12 samples in duplicates

- ✓ For measurement in duplicates, add 20µL of extract into 4 wells as shown below for samples S1-S11. Add 20µL of water into empty wells (A1, A2, A3 and A4) for negative controls. We recommend to run positive control in parallel by adding 20µL of 2.5mM PRPP solution (1.25mg/ml) into D5, D6, D7 and D8 wells (sample S11).



- ✓ Add 180µL of **"Reaction mixture BLANK"** per well (total volume 200µL) and 180µL of **"Reaction mixture Hx+"** as shown below:



- ✓ Agitate the plate for 2 min and incubate it for 30h at room temperature
- ✓ Program plate reader for absorbance reading (absorbance at 340nm), Insert the plate into the reader, agitate for 1min and read absorbance data at the end of incubation..

VI Calculation of PRPP concentration

$$[\text{PRPP}] \text{ in the sample (in µM)} = \frac{\text{Absorbance}_{\text{Hx+}} - \text{Absorbance}_{\text{Blank}}}{4.9}$$

where: 4.9 is extinction coefficient of 1mM NADH at 340nm corrected for l (path-length of round-bottom 200µL-well 96-well plate Corning, Costar®, ref. 3797) as measured with iEMS Labsystems plate reader (Thermo).