

## Continuous PRPP-S Assay Kit

### Product Information

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**Cat.No.** Kit-0871

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### Product Overview

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PRPP-S Assay Kit is designed for continuous monitoring of PRPP synthesis. The assay is based on coupling of two recombinant enzymes : Hypoxanthine-guanine phosphoribosyltransferase (HGPRT) and Inosine Monophosphate Dehydrogenase (IMPDH).

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

- (1) In the presence of ATP and P-ribose, PRPP-Synthetase enzyme catalyzes the formation of PRPP.
- (2) In the presence of Hypoxanthine (Hx), PRPP is converted to IMP by Hypoxanthine-guanine phosphoribosyltransferase (HGPRT).
- (3) IMP is immediately oxidized by a highly active IMPDH in the presence of NAD with simultaneous formation of NADH<sub>2</sub> directly monitored spectrophotometrically at 340nm.

The assay is developed for measuring PRPP-S activity in vitro or in cell lysates. For maximal accuracy, the assays with cell lysates are run with and without P-ribose in parallel. The absorbance rate observed in the absence of inosine is used as blank and is subtracted from the absorbance rate measured in its presence.

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### Storage

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The kit is shipped at room temperature since dry reagents and lyophilized enzymes are stable at room temperature (up to 2 weeks). However, for long time storage the kit should be frozen upon arrival and stored at -20°C.

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### Size

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24 analysis

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### Kit Components

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Cysteine (12mg, powder);  
NAD (17mg, powder)  
ATP (33mg, powder)  
P-ribose (10mg, powder)  
HPRT and IMPDH enzymes, lyophilized

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Reaction buffer (glass vial, 10mL) ;

one transparent 96-well plate

Once dissolved, the reagents can be stored at -20°C for three months.

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### Materials Required but Not Supplied

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- 1) Plate agitator
- 2) Plate reader fitted with a filter 340nm

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### Assay Protocol

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Preparation of 1ml "Reaction mixture"

IMPORTANT: Use only autoclaved Milli-Q water to inactivate ubiquitous phosphatases and to avoid dephosphorylation of P-ribose and PRPP present in reaction mixture

1. Shortly spin the tubes before opening to recover the powder at the bottom;
2. Thaw "Reaction buffer" (do not heat); equilibrate at room temperature ;
3. Add 200µL of deionized water to the tube with "HPRT and IMPDH enzymes", agitate (do not vortex to avoid foam) and spin shortly;
4. Add 100µL of deionized water to each of four tubes (Cysteine, NAD, ATP and P-ribose). Vortex until complete dissolution, spin shortly;
5. Put 0.85mL of reaction buffer in a clean 1.5mL tube, add 9µL of "Cysteine", 9µL of "NAD" and 9µL of "ATP" solutions. Do not add P-ribose solution
6. Close and agitate by inverting;
7. Add 18µL of "HPRT-IMPDH enzyme" solution; close and agitate by inverting, spin shortly;

Composition of reaction mixture: 100mM Tris-HCl, 100mM KCl, 12mM MgCl<sub>2</sub>, BSA 1mg/ml, 2,5mM NAD, 7,5mM cysteine, 4mM ATP, IMPDH-HGPRT 30mU/ml each, pH8.5, start by P-ribose (3.5mM).

Reaction monitoring

1. Program plate reader for kinetics absorbance reading (every 1 min), 37°C.
2. Add desired amount of PRPP-S solution (1-10µL) per well to four wells, followed by addition of 200µL of "Reaction mixture"
3. Insert the plate into the reader pre-heated at 37°C, agitate for 1 min and incubate for 15 min;



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4. To start the reaction, add 2µL of P-ribose solution to two wells (two other will be used as Blank), agitate and monitor the reaction at 340nm at 37°C for 30 min-1 hour with data collection every min.

### Analysis

1. Calculate the absorbance rate per hour for reaction buffers with Ribose 5-phosphate (ARP5R) and without (ARblank).

2. Calculate Mean ARP5r and Mean ARblank

3. Calculate PRPP-S activity using following formula:

Activity= (Mean ARP5r-Mean ARblank) / (4.9x[Hgb]) x1000 = (0.229-0.014)/(4.9x0.62)x1000 =71 nmol/hour/mg of Hgb

where: Mean ARP5r=0.229;

Mean ARblank=0.014;

[Hgb], final haemoglobin concentration used in assay=0.62mg/ml.

4.9 is the absorbance of 1mM NADH at 340nm in 200µL-round-bottom well of 96-well microplate.