Continuous PRPP-S Assay Kit

Cat. No. Kit-0871 **Lot. No.** (See product label)

SPECIFICATION

Product Overview	PRPP-S 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). 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 NADH2 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.
Description	PRPP-S is an enzyme that converts ribose 5-phosphate into phosphoribosyl pyrophosphate (PRPP). It is classified under EC 2.7.6.1. The enzyme is involved in the synthesis of nucleotides (purines and pyrimidines), cofactors NAD and NADP, and amino acids histidine and tryptophan, linking these biosynthetic processes to the pentose phosphate pathway, from which the substrate ribose 5-phosphate is derived. Ribose 5-phosphate is produced by the HMP Shunt

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	Pathway from Glucose-6-Phosphate. The product phosphoribosyl pyrophosphate acts as an essential component of the purine salvage pathway and the de novo synthesis of purines. Dysfunction of the enzyme would thereby undermine purine metabolism. Ribose-phosphate pyrophosphokinase exists in bacteria, plants, and animals, and there are three isoforms of human ribose-phosphate pyrophosphokinase. In humans, the genes encoding the enzyme are located on the X chromosome.
Storage	Once dissolved, the reagents provided in the kit are not stable and should be stored on ice and used the day of preparation.
Size	24 analysis
Kit Components	The kit allows to perform 24 analysis in a time (12 samples in duplicate or 8 samples in triplicate). A standard PRPP-S Assay Kit: one tube "Cofactor 1" (DTT); one tube "Cofactors 2 & 3" (NAD and ATP); one tube "IMPDH-HGPRT enzymes"; one vial "Blank" (pre-filled with 10mL of "Reaction buffer"); one transparent 96-well plate.
Materials Required but Not Supplied	1) Plate agitator; 2) Plate reader fitted with a filter 340nm, Epoch; PerkinElmer. 3) D-Ribose 5-phosphate. Important: Since P-ribose solutions in water are unstable because of ubiquitous presence of phosphatases, we recommend preparing the tubes with indicated mg of P-ribose, storing as a powder at -20°C and dissolving at very last moment.
Preparation	1. Reconstitute the enzymes with 200µ of deionized water to the tubes with. Agitate "IMPDH-HGPRT enzymes" until complete dissolution of the powder.

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	2. Quantitatively transfer the content of 4 tubes with "Cofactor 1", "Cofactor 2&3" and "IMPDH-HGPRT enzymes" to "Blank" tube To do so: - pipet 1ml of buffer from "Blank" 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 "Blank" 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. 3. Weight 10mg of P-Ribose in a clean labeled tube (15ml). 4. Add 5ml of "Blank" to 10mg of P-ribose. You have prepared: 5ml of "Blank"; 5ml of "Reaction mixture with P-Ribose".
Assay Protocol	1. Preparation of hemolysates. The pellet of PBS-washed erythrocytes from 100µ of blood was frozenthawed twice, resuspended in 1mL of ice-cold deionized water and used directly for PRPP-S quantification. 2. Add 4µ of hemolysates per well. 3. Add 200µ of "Blank" per well and 200µ of "Reaction mixture" containing 1mM P-ribose. 4. Program plate reader for kinetics absorbance reading (every 1 min), 37°C. Insert the plate into the reader pre-heated at 37°C, agitate for 1min and monitor the reaction at 340nm at 37°C for 1 hour with data collection every 2min.
Analysis	1. Calculate the absorbance rate per hour for reaction buffers with Ribose 5-phosphat e (ARP5R) and without (ARblank). 2. Calculate Mean ARP5r and Mean ARblank. 3. Measure the concentration of hemoglobin [Hgb] in hemolysates using Drabkin's rea gent and calculate final [Hgb] concentration used in assay. 4. PRPP-S activity is calculated by the following formula: Activity= (MeanARP5r-MeanARblank)/(4.9x[Hgb])x103 = (0.229-0.014)/(4.9x0.62)x10 3 =71 nmol/hour/mg of Hgb

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	where: MeanARP5r=0.229; MeanARblank=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.

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