

## PRECICE® HPRT Assay Kit Hypoxanthine-guanine phosphoribosyl transferase Assay Kit

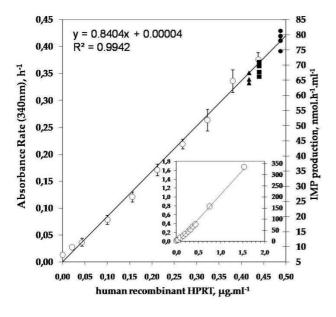
PRECICE® HPRT Assay kit provides a simple non-radioactive assay for measurement of Hypoxanthine-guanine phosphoribosyltransferase (HPRT) activity in a convenient 96-well plate format. In the assay, HPRT activity is measured as a rate of production of IMP, which is oxidized by recombinant IMP-dehydrogenase with concomitant formation of NADH<sub>2</sub>. The formation of NADH<sub>2</sub> is continuously monitored spectrophotometrically at 340nm. The assay is linear up to 340nmol/hour/ml of HPRT activity, enables the detection of allow as 6.75nmol/hour/ml and provides results with uncertainties of 6.5%. Specific activities of HPRT in lysates of erythrocytes and cultured cells measured by PRECICE® HPRT Assay kit are comparable to those obtained previously by radiochemical procedure.

## Principle

IMP-dehydrogenase enzyme catalyzes the irreversible oxidation of IMP to XMP with simultaneous reduction of NAD to NADH<sub>2</sub>. **PRECICE**® **HPRT Assay Kit** takes advantage of this property for monitoring the activity of HPRT. In the assay, HPRT activity is measured as a rate of production of IMP, which is immediately oxidized to XMP by IMPDH with concomitant reduction of NAD to NADH<sub>2</sub> measurable by absorbance at 340nm.

Accurate estimation of HPRT activity with in NOVOCIB's IMPDH-based assay is done:

- in unidirectional reaction where IMP produced is immediately converted by IMPDH to XMP;
- through continuous monitoring of HPRT activity at 340nm in a convenient 96-well plate;
- by simultaneous analysis of 15 samples in triplicate per plate (2h);



## Calibration curve of IMPDH-based PRECICE® HPRT Assav

The rate of the increase in absorbance at 340nm per hour as a function of the concentration of human recombinant HPRT enzyme (NovoCIB, ref. E-Nov-9). The changes in absorbance corresponding to 3 different control hemolysates diluted in complete reaction mixture to final hemoglobin concentration 1mg/ml (n=4) are shown by filled squares, filled triangles and filled circles. The insert shows a linear correlation observed in whole range of 21ng/ml up to 1.5µg/ml of recombinant HPRT; the units are the same.

**Kit Content:** 

- Bacterial IMPDH
- IMPDH Reconstitution Buffer
- NAD 100x
- DTT
- HKM Buffer containing hypoxanthine (10x)