# PRECICE® HPRT Assay Kit

## *Hypoxanthine-guanine phosphoribosyltransferase Assay Kit*

**For research use only. Not for use in diagnostic procedures**

## I. Background

Hypoxanthine phosphoribosyltransferase is a purine salvage enzyme that catalyzes the reversible transfer of the 5-phosphoribosyl moiety from α–D-5-phosphoribosyl-1-pyrophosphate (PRPP) to a purine base (hypoxanthine or guanine) to form a nucleoside monophosphate (inosine monophosphate or guanosine monophosphate, respectively). In the presence of pyrophosphate, HPRT enzyme catalyzes also the hydrolysis of IMP and GMP, although this reverse reaction is much less favored than forward one. Human HPRT enzyme does not hydrolyse XMP.

*HPRT1* gene is one of the best characterized in the human genome for two reasons: (i) *HPRT1* gene is widely used as a somatic cell genetic marker in genotoxicity / mutagenicity studies; (ii) the defects within the human enzyme are associated with inherited gouty arthritis and Lesch–Nyhan syndrome and more than 300 disease-associated mutations in human *HPRT1* gene leading to partial or complete deficiencies of the HPRT enzyme have been described[1]. In view of the high variability of HPRT1 gene, a rapid biochemical assay would be useful both for basic science and clinical research.

In addition, since most parasitic protozoan are obligate auxotrophs of purines and entirely depend therefore on their purine salvage pathways, protozoan HPRT enzyme is an attractive target for the discovery of new anti-parasitic drugs[2]. The enzymatic microplate assay enabling monitoring of HPRT activity may therefore accelerate the search of new anti-parasitic drugs.

## II. Principle

**PRECICE® HPRT Assay Kit** provides an enzymatic tool for continuous spectrophotometric monitoring of 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 IMPDH enzyme with simultaneous reduction of $NAD^+$ to NADH measurable by absorbance at 340nm (Fig. 1).

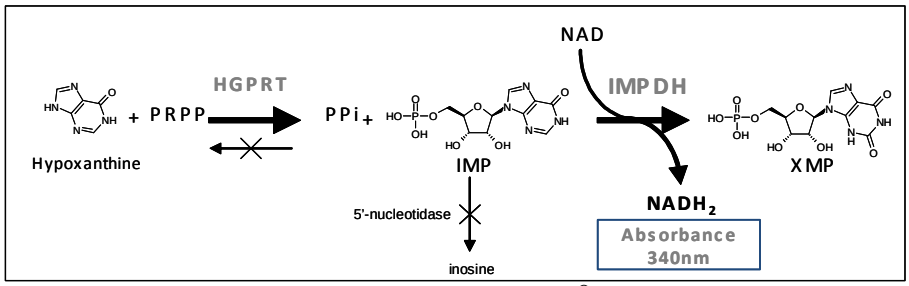


**Figure 1. Enzymatic principle of PRECICE® HPRT Assay Kit.**

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

## III. Kit Contents

A standard 100-assay PRECICE® HPRT Assay Kit contains

- vial of **"Recombinant IMPDH" (500mU)**
- vial of **"Enzyme Buffer" (500µL)**
- vial of **"NAD" (80mg, to be dissolved in 200µL of deionized water, store at -80°C after reconstitution)**
- vial of **"TKM Buffer 13,33x " (1,5mL)**

**Optional:**

- vial of "**Human Recombinant HPRT**" for preparing enzyme solution at 75mU/ml
- Transparent 96-well plate (round-bottom 96-well plate Corning, Costar®, ref. 3797)

**Not provided:**

DTT (DL-dithiothreitol)

PRPP (α–D-5-phosphoribosyl-1-pyrophosphate, available at Sigma-Aldrich, ref. P8296)

**IMPORTANT:**
**The following instructions are given to measure the activity of HPRT enzyme, in a range allowing this measurement by spectrophotometry as described here below.**
**NovoCIB does not guarantee the use of its PRECICE® HPRT Assay Kit or of one or several of its components, in other conditions than those described in this user manual and/or for other purpose than R&D.**

# IV. Experimental Protocols

## 1. Reconstitution of IMPDH enzyme

Immediately prior to prepare the standard reaction buffer, reconstitute the lyophilized recombinant IMP-dehydrogenase (IMPDH) in 250µL of **"Enzyme Buffer"** and mix until dissolved. Once the desired quantity of enzyme has been pipetted for the preparation of standard reaction buffer, store the enzyme at -20°C .

## 2. Preparation of standard reaction buffer (1x)

Standard reaction buffer is prepared according to the following instructions. Please, note that the total volume of standard reaction buffer prepared will be split in two equal volumes, in two tubes. Only one of them will contain PRPP, the other remaining without PRPP (used for blank controls).

**For a 20mL total volume, sufficient for one 96-well plate:**

**i)** Weight 15.4mg of DTT in a clean tube labeled "DTT" and add 100µL of $dH_20$ to prepare 1M DTT solution. Agitate until dissolved. ***Important: DTT solution must be freshly prepared, since DTT in aqueous solution is highly unstable with half-life ~10h. Never use old solutions of DTT.***

**ii)** Prepare a 500mM NAD solution by adding 200µL of deionized water to "NAD" tube containing 80mg of NAD powder. Agitate.

**iii)** Transfer **in the following order** the content of vial **"TKM 13,33x", "DTT", "NAD"** and **"Recombinant IMPDH"** into a tube containing 18mL of $dH_2O$.

**iv)** In a clean labeled 15mL tube, weight 10mg of PRPP (Sigma-Aldrich, ref. P8296).

**v)** Transfer 10mL of the standard reaction buffer obtained in iii) into the tube containing PRPP.

**vi)** Incubate both tubes at 37°C for 5-10min.

**Alternatively, for other volume**, prepare standard reaction buffer so that 1mL contain:

- 900µL of $dH_20$
- 75µL "TKM 13,33x"
- 5µL of 1M DTT
- 10µL of 500mM NAD
- 10µL of recombinant IMPDH
- (when added, 0.5mg PRPP)

## 3. Validation of the experimental protocol with purified human recombinant HPRT enzyme

### 3.1. Reconstitution of human recombinant HPRT enzyme

Immediately prior to use, reconstitute the lyophilized human recombinant HPRT enzyme with the volume of "Enzyme Buffer" indicated on the label, to get a 75mU/ml solution and mix until dissolved. Once the desired quantity of enzyme has been pipetted for the preparation of the assay, store the enzyme at -20°C.

### 3.2. Measurement of HPRT activity with microplate reader

**i)** Add 4µL of reconstituted human recombinant HPRT per well in replicate of six (finale concentration 1.5mU/ml). Add 196µL of standard reaction buffer free of PRPP to three of them (blank) and 196µL of reaction buffer containing PRPP to three others.

**ii)** Insert the plate into the reader pre-heated at 37°C , agitate for 2min and monitor the reaction at 340nm at 37°C for 2h with data collection every 5min. Typical results obtained with 1.5mU/ml of purified HPRT in the presence of 2mM PRPP or its absence are shown on Table 1 and Figure 1.

### 3.3. Calculation of HPRT activity

**i)** For the first two hours, calculate the absorbance rate per hour for reaction buffer with 2mM PRPP ($AR_{PRPP}$) and without PRPP ($AR_{Blank}$).

**ii)** Calculate $\overline{AR}_{PRPP}$ and $\overline{AR}_{Blank}$, respectively the mean of $AR_{PRPP}$ measurements and the mean of $AR_{Blank}$ measurements.

**iii)** Calculate HPRT activity as follows:

$$\textbf{HPRT Activity (in nmol /ml/ hour)} = \frac{\overline{AR}_{PRPP} - \overline{AR}_{Blank}}{\varepsilon \cdot l} \times 10^6$$

where: ε is the molar extinction coefficient of NADH at 340nm : ε = 6220 $M^{-1}.cm^{-1}$
l is the path-length, expressed in cm. l = 0.789 for a 200µL- round-bottom well of 96-well microplate (Corning, Costar®, ref. 3797)

In our example, according to the data of Table 1:

$\overline{AR}_{PRPP}$ = 0.500 $h^{-1}$ and $\overline{AR}_{Blank}$ = 0.038 $h^{-1}$

$$\text{HPRT Activity (in nmol /mL / hour)} = \frac{0.500 - 0.038}{6220 \cdot 0.789} \times 10^6 = \textbf{94.16 nmol / mL / h}$$

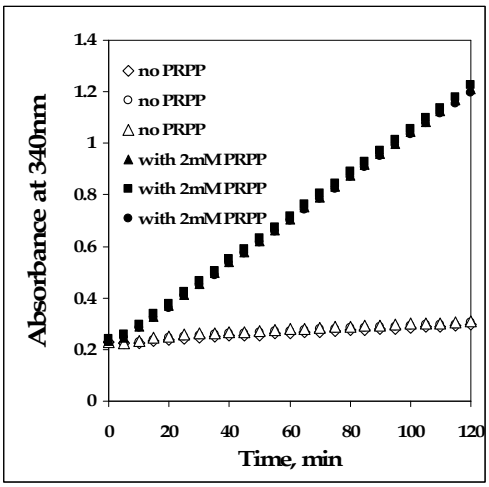


**Figure 1. Time course of IMP formation by human recombinant HPRT (1.5mU/ml) incubated in the presence of PRPP in standard reaction buffer or its absence. After vigorous shaking for 2min, the absorbance at 340nm was monitored at 37°C using iEMS Plate Reader (Thermo Scientific) and round-bottom 96-well microplate (Corning, Costar®, ref. 3797).**

**Table 1.**

| Time, min | Absorbance at 340nm without PRPP | | | Absorbance at 340nm with 2mM PRPP | | |
|---|---|---|---|---|---|---|
| **0** | 0.228 | 0.225 | 0.227 | **0.232** | **0.24** | **0.227** |
| **5** | 0.224 | 0.222 | 0.225 | **0.248** | **0.258** | **0.244** |
| **10** | 0.226 | 0.228 | 0.235 | **0.287** | **0.298** | **0.283** |
| **15** | 0.232 | 0.239 | 0.245 | **0.328** | **0.338** | **0.323** |
| **20** | 0.237 | 0.245 | 0.251 | **0.369** | **0.378** | **0.362** |
| **25** | 0.241 | 0.251 | 0.257 | **0.413** | **0.424** | **0.407** |
| **30** | 0.247 | 0.255 | 0.261 | **0.455** | **0.465** | **0.449** |
| **35** | 0.249 | 0.258 | 0.264 | **0.495** | **0.506** | **0.49** |
| **40** | 0.253 | 0.261 | 0.268 | **0.539** | **0.55** | **0.533** |
| **45** | 0.253 | 0.262 | 0.268 | **0.577** | **0.588** | **0.571** |
| **50** | 0.256 | 0.266 | 0.272 | **0.621** | **0.634** | **0.616** |
| **55** | 0.261 | 0.27 | 0.276 | **0.663** | **0.675** | **0.657** |
| **60** | 0.264 | 0.272 | 0.278 | **0.705** | **0.718** | **0.699** |
| **65** | 0.267 | 0.275 | 0.282 | **0.749** | **0.763** | **0.743** |
| **70** | 0.269 | 0.278 | 0.284 | **0.791** | **0.804** | **0.783** |
| **75** | 0.272 | 0.281 | 0.287 | **0.833** | **0.846** | **0.825** |
| **80** | 0.275 | 0.284 | 0.29 | **0.875** | **0.89** | **0.868** |
| **85** | 0.277 | 0.286 | 0.292 | **0.917** | **0.931** | **0.91** |
| **90** | 0.279 | 0.288 | 0.294 | **0.957** | **0.972** | **0.949** |
| **95** | 0.281 | 0.29 | 0.296 | **0.999** | **1.015** | **0.992** |
| **100** | 0.285 | 0.294 | 0.3 | **1.042** | **1.058** | **1.034** |
| **105** | 0.289 | 0.296 | 0.302 | **1.083** | **1.099** | **1.076** |
| **110** | 0.289 | 0.297 | 0.303 | **1.123** | **1.139** | **1.115** |
| **115** | 0.291 | 0.299 | 0.306 | **1.165** | **1.181** | **1.156** |
| **120** | 0.296 | 0.304 | 0.31 | **1.207** | **1.224** | **1.198** |
| Absorbance rate per hour | 0.036 | 0.039 | 0.039 | **0.499** | **0.503** | **0.497** |

## 4. Measurement of HPRT activity in hemolysates

### 4.1. Preparation of hemolysates

This protocol has been developed with erythrocytes purified from 1mL of peripheral blood using Ficoll-Hypaque gradient and washed once with PBS. The pellet of washed packed erythrocytes was resuspended in 4mL of ice-cold $dH_20$ and sonicated for 1min on ice (Sonopuls, Bandelin, 20% cycle, 50% power). The sonicated hemolysate was immediately used for Haemoglobin measurement and HPRT activity assay, without additional centrifugation.

Keep 10 to 20µL of the supernatant for Haemoglobin measurement and use the remaining for HPRT activity assay.

### 4.2. Measurement of HPRT activity with microplate reader

**Preamble:** Since hemolysates show inherent absorbance at 340nm, we strongly recommend to check the initial absorbance of diluted hemolysate at 340nm **before** starting HPRT quantification. To do so, pipet 2, 4, and 6µL of hemolysate into three wells of a 96-well plate and add 198, 196 and 194µL (qsp 200µL) of deionized water, respectively. Agitate for 2min and read the absorbance at 340nm. Use the volume of hemolysate that provides absorbance within a range of 0.9-1.1. It usually corresponds to 4µL of hemolysate per well.

**i)** Pipet 4µL (or 2, or 6: see Preamble above) of hemolysate into a well, in replicates of six, and add 196µL (qsp200µL) of standard reaction buffer free of PRPP to three of them (blank) and 196µL (qsp200µL) of reaction buffer containing PRPP to three others.

**ii)** Insert the plate into the reader pre-heated at 37°C , agitate for 2min and monitor the reaction at 340nm at 37°C for 2h with data collection every 5min. Typical results obtained with 6µL of hemolysates in the presence of 2mM PRPP or in its absence are shown on Figure 2 and Table 2.

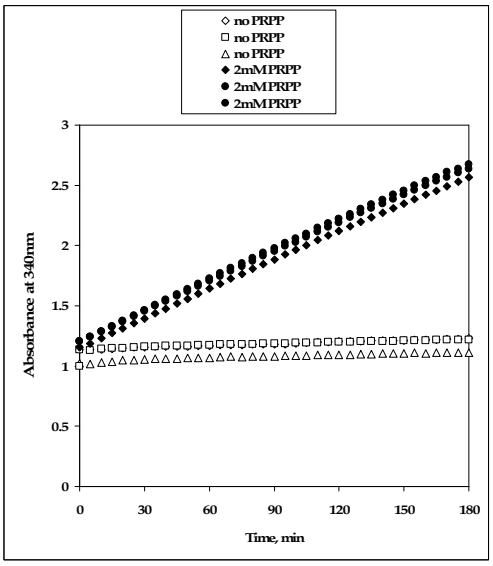


**Figure 2. Kinetics of IMP formation in hemolysates incubated in the presence of PRPP (Sigma-Aldrich, ref. P8296) in standard reaction buffer. After vigorous shaking for 2min, the absorbance at 340nm was monitored at 37°C using iEMS Plate Reader (Thermo Scientific) and round-bottom 96-well microplate (Corning, Costar®, ref. 3797).**

**Table 2**

| Time, min | Absorbance at 340nm without PRPP | | | Absorbance at 340nm with 2mM PRPP | | |
|---|---|---|---|---|---|---|
| **0** | 1.139 | 1.139 | 1.02 | **1.156** | **1.205** | **1.205** |
| **5** | 1.135 | 1.132 | 1.019 | **1.186** | **1.242** | **1.24** |
| **10** | 1.138 | 1.141 | 1.028 | **1.23** | **1.289** | **1.284** |
| **15** | 1.143 | 1.146 | 1.037 | **1.271** | **1.332** | **1.326** |
| **20** | 1.149 | 1.15 | 1.046 | **1.311** | **1.375** | **1.369** |
| **25** | 1.153 | 1.156 | 1.051 | **1.353** | **1.42** | **1.411** |
| **30** | 1.156 | 1.16 | 1.055 | **1.396** | **1.464** | **1.453** |
| **35** | 1.159 | 1.163 | 1.058 | **1.436** | **1.508** | **1.498** |
| **40** | 1.16 | 1.166 | 1.061 | **1.477** | **1.551** | **1.539** |
| **45** | 1.162 | 1.167 | 1.063 | **1.517** | **1.593** | **1.58** |
| **50** | 1.164 | 1.169 | 1.064 | **1.559** | **1.638** | **1.622** |
| **55** | 1.168 | 1.174 | 1.07 | **1.603** | **1.683** | **1.665** |
| **60** | 1.168 | 1.174 | 1.07 | **1.642** | **1.724** | **1.704** |
| **65** | 1.171 | 1.178 | 1.074 | **1.685** | **1.769** | **1.746** |
| **70** | 1.173 | 1.18 | 1.077 | **1.726** | **1.813** | **1.79** |
| **75** | 1.174 | 1.18 | 1.075 | **1.763** | **1.851** | **1.827** |
| **80** | 1.177 | 1.183 | 1.079 | **1.806** | **1.896** | **1.873** |
| **85** | 1.178 | 1.186 | 1.082 | **1.846** | **1.938** | **1.913** |
| **90** | 1.179 | 1.187 | 1.082 | **1.885** | **1.977** | **1.954** |
| **95** | 1.181 | 1.188 | 1.084 | **1.926** | **2.022** | **1.995** |
| **100** | 1.184 | 1.191 | 1.086 | **1.963** | **2.059** | **2.03** |
| **105** | 1.194 | 1.191 | 1.085 | **2.002** | **2.099** | **2.071** |
| **110** | 1.199 | 1.195 | 1.091 | **2.044** | **2.145** | **2.116** |
| **115** | 1.201 | 1.196 | 1.092 | **2.083** | **2.183** | **2.155** |
| **120** | 1.203 | 1.197 | 1.091 | **2.119** | **2.221** | **2.19** |
| *Absorbance per hour* | *0.031* | *0.031* | *0.034* | ***0.488*** | ***0.512*** | ***0.497*** |

### 4.3. Calculation of HPRT activity

**i)** Measure the concentration of haemoglobin [Hgb] in sonicated hemolysates using Drabkin's reagent and calculate final [Hgb] concentration used in the assay.

**ii)** For the first two hours, calculate the absorbance rate per hour for reaction buffer with 2mM PRPP ($AR_{PRPP}$) and without PRPP ($AR_{Blank}$).

Calculate the means $\overline{AR}_{PRPP}$ and $\overline{AR}_{Blank}$

**iii)** HPRT activity is calculated by the following formula:

$$\textbf{HPRT Activity (in nmol /mg of haemoglobin / hour)} = \frac{\overline{AR}_{PRPP} - \overline{AR}_{Blank}}{\varepsilon \cdot l \cdot [Hgb]} \times 10^6$$

where: ε is the molar extinction coefficient of NADH at 340nm : ε = 6220 $M^{-1}.cm^{-1}$
l is the path-length, expressed in cm. l = 0.789cm for a 200µL- round-bottom well of 96-well microplate (Corning, Costar®, ref. 3797)
[Hgb] is the final concentration of haemoglobin used in the assay, expressed in mg/mL

In our example, according to the data of Table 2:

$\overline{AR}_{PRPP}$ = 0.499 $h^{-1}$ and $\overline{AR}_{Blank}$ = 0.032 $h^{-1}$ **[Hgb]** = 1.1 mg/mL

$$\text{HPRT Activity (in nmol / mg Haemoglobin / hour)} = \frac{0.499 - 0.032}{6220 \cdot 0.789 \cdot 1.1} \times 10^6 = \textbf{86.52 nmol / mg Hgb / h}$$

## 5. Measurement of HPRT activity in peripheral blood mononuclear cells (PBMC)

### 5.1. Preparation of lysates of PBMC

This protocol has been developed with PBMC purified from 1.25mL of peripheral blood using Ficoll-Hypaque gradient and washed once with PBS. The pellet of washed PBMC was resuspended in 1mL of ice-cold $dH_20$ and sonicated for 1min on ice (Sonopuls, Bandelin, 20% cycle, 50% power). The sonicated extract was centrifuged for 1min at 13,000g at 4°C. The supernatant was used for protein measurement and HPRT activity assay.

Keep 20 to 50µL of the supernatant for protein measurement and use the remaining for HPRT activity assay.

### 5.2. Preparation of 2-fold concentrated reaction buffer (2x)

A 2-fold concentrated buffer is required to measure HPRT activity from lysates of PBMC. As described in section 2, the total volume of 2-fold concentrated standard reaction buffer prepared will be split in two equal volumes, in two tubes. Only one of them will contain PRPP, the other remaining without PRPP (used for blank controls).

2-fold concentrated standard reaction buffer is prepared according to the following instructions.

**For a 10mL total volume, sufficient for one 96-well plate:**

**i)** Weight 15.4mg of DTT in a clean tube labeled "DTT" and add 100µL of $dH_20$ to prepare 1M DTT solution. Agitate until dissolved. ***Important:*** ***DTT solution must be freshly prepared, since DTT in aqueous solution is highly unstable with half-life ~10h. Never use old solutions of DTT.***

**ii)** Prepare a 500mM NAD solution by adding 200µL of deionized water to "NAD" tube containing 80mg of NAD powder. Agitate.

**iii)** Transfer **in the following order** the content of vial **"TKM 13,33x", "DTT", "NAD"** and **"Recombinant IMPDH"** into a tube containing 8mL of $dH_2O$.

**iv)** In a clean labeled 10mL tube, weight 10mg of PRPP (Sigma-Aldrich, ref. P8296).

**v)** Transfer 5mL of the standard reaction buffer obtained in iii) into the tube containing PRPP.

**vi)** Incubate both tubes at 37°C for 5-10min.

**Alternatively, for other volume**, prepare 2-fold concentrated standard reaction buffer so that 1mL contain:

- 800µL of $dH_20$
- 150µL "TKM 13,33x"
- 10µL of 1M DTT
- 20µL of 500mM NAD
- 20µL of recombinant IMPDH
- (when added, 1mg PRPP)

### 5.3. Measurement of HPRT activity with microplate reader

**i)** Pipet 100µL of PBMC lysate into a well, in replicates of six, and add 100µL of 2-fold concentration reaction buffer free of PRPP to three of them (blank) and 100µL of 2-fold concentration reaction buffer containing PRPP to three others. To provide meaningful data, final protein concentration in the assay with PBMC extract should be >0.15mg/ml.

**ii)** Insert the plate into the reader pre-heated at 37°C , agitate for 2min and monitor the reaction at 340nm at 37°C for 4h with data collection every 15min. Typical results obtained with two different PBMC lysates in the presence of 2mM PRPP or in its absence are shown on Figure 3 and Table 3.

### 5.4. Calculation of HPRT activity

**i)** Measure the concentration of protein [Protein] used in the assay.

**ii)** Calculate the absorbance rate per hour for reaction buffer with 2mM PRPP ($AR_{PRPP}$) and without PRPP ($AR_{Blank}$).
Calculate the means $\overline{\mathbf{AR}}_{\mathbf{PRPP}}$ and $\overline{\mathbf{AR}}_{\mathbf{Blank}}$

**iii)** HPRT activity is calculated by the following formula:

$$\textbf{HPRT Activity (in nmol / mg Protein / hour)} = \frac{\overline{\mathbf{AR}}_{\mathbf{PRPP}} - \overline{\mathbf{AR}}_{\mathbf{Blank}}}{\boldsymbol{\varepsilon} \,.\, \mathbf{l} \,.\, \mathbf{[Protein]}} \times 10^6$$

where: ε is the molar extinction coefficient of NADH at 340nm : ε = 6220 $M^{-1}.cm^{-1}$
l is the path-length, expressed in cm. l = 0.789cm for a 200µL- round-bottom well of 96-well microplate (Corning, Costar®, ref. 3797)
[Protein] is the final concentration of protein used in the assay, expressed in mg/mL

In our example, according to the data of Table 3:

$\overline{\mathbf{AR}}_{\mathbf{PRPP}}$ = 0.171 $h^{-1}$ and $\overline{\mathbf{AR}}_{\mathbf{Blank}}$ = 0.045 $h^{-1}$ **[Protein]** = 0.166 mg/mL

$$\text{HPRT Activity (in nmol / mg Protein / hour)} = \frac{0.171 - 0.045}{6220 \,.\, 0.789 \,.\, 0.166} \times 10^6 = \textbf{154.6 nmol / mg Protein / h}$$

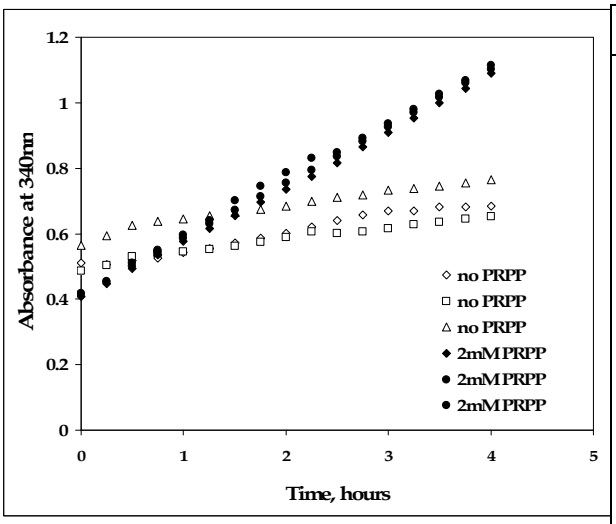


**Figure 3. Figure 3. The formation of NADH in the lysate of PBMC incubated in the presence of PRPP in standard reaction buffer (final protein concentration 0.166mg/ml). After shaking for 2min, the absorbance at 340nm was monitored at 37℃ using iEMS Plate Reader (Thermo Scientific) and round-bottom 96-well microplate (Corning, Costar®, ref. 3797).**

**Table 3.**

| Time, min | Absorbance at 340nm without PRPP | | | Absorbance at 340nm with 2mM PRPP | | |
|---|---|---|---|---|---|---|
| **0** | 0.512 | 0.486 | 0.564 | **0.408** | **0.415** | **0.419** |
| **15** | 0.507 | 0.504 | 0.595 | **0.448** | **0.451** | **0.454** |
| **30** | 0.519 | 0.530 | 0.625 | **0.494** | **0.502** | **0.510** |
| **45** | 0.525 | 0.539 | 0.638 | **0.535** | **0.543** | **0.551** |
| **60** | 0.542 | 0.546 | 0.646 | **0.576** | **0.586** | **0.596** |
| **75** | 0.555 | 0.553 | 0.654 | **0.616** | **0.63** | **0.640** |
| **90** | 0.572 | 0.563 | 0.665 | **0.656** | **0.673** | **0.702** |
| **105** | 0.587 | 0.574 | 0.675 | **0.696** | **0.714** | **0.745** |
| **120** | 0.602 | 0.589 | 0.685 | **0.735** | **0.755** | **0.787** |
| **135** | 0.620 | 0.605 | 0.699 | **0.775** | **0.795** | **0.830** |
| **150** | 0.640 | 0.601 | 0.711 | **0.816** | **0.837** | **0.849** |
| **165** | 0.657 | 0.605 | 0.719 | **0.864** | **0.882** | **0.891** |
| **180** | 0.669 | 0.617 | 0.732 | **0.909** | **0.927** | **0.936** |
| **195** | 0.670 | 0.627 | 0.738 | **0.954** | **0.971** | **0.980** |
| **210** | 0.682 | 0.635 | 0.745 | **1.000** | **1.017** | **1.026** |
| **225** | 0.681 | 0.644 | 0.755 | **1.044** | **1.060** | **1.069** |
| **240** | 0.684 | 0.653 | 0.764 | **1.089** | **1.102** | **1.114** |
| Absorbance rate per hour | 0.052 | 0.039 | 0.045 | **0.169** | **0.172** | **0.173** |

## References:

[1] Torres, R. J.,Puig, J. G., Hypoxanthine-guanine phosphoribosyltransferase (HPRT) deficiency: Lesch-Nyhan syndrom. *Orphanet J Rare Dis.* **2**, 48-57 (2007).

[2] Datta, A.K, Datta, R., Sen, B. Antiparasitic chemotherapy: tinkering with the purine salvage pathway. *Adv. Exp. Med. Biol.* **625**, 116-132 (2008).