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BioVision
BioVision Incorporated

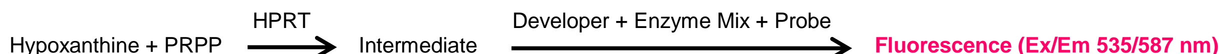
PicoProbe™ Hypoxanthine Phosphoribosyl Transferase Activity Assay Kit (Fluorometric)

06/18

(Catalog # K478-100; 100 assays; Store at - 20ºC)

I. Introduction:

Hypoxanthine Phosphoribosyl Transferase or HPRT (EC 2.4.2.8) plays an important role in the generation of purine nucleotides through the purine salvage pathway and is present in prokaryotes and eukaryotes. The enzyme converts guanine to guanosine monophosphate and hypoxanthine to inosine monophosphate by transferring a phosphoribosyl group from phosphoribosyl pyrophosphate (PRPP) to the purine molecule, and releases an inorganic pyrophosphate molecule in the process. Mutations in HPRT lead to developmental disorders such as Lesch Nyhan Disease (LND), characterized by neurological and behavioral abnormalities. Its deficiency also leads to defective basal ganglia expression of the neurotransmitter dopamine and aberrant neuronal function. This causes dysregulation of multiple dopamine- related developmental functions and cellular signaling defects. BioVision's HPRT Activity Assay Kit is a simple one-step plate based assay kit for the measurement of HPRT activity in biological samples. HPRT catalyzes the conversion of hypoxanthine to inosine monophosphate (IMP). IMP then undergoes a series of enzymatic reactions to convert a non-fluorescent probe to a fluorescent product. The signal, which is proportional to the generated product can be read in kinetic mode at Ex/Em 535/587 nm. The assay can detect as low as 2 mU HPRT.



II. Applications:

- Measurement of HPRT activity in cell and tissue lysates using a 96-well plate format

III. Sample Type:

- Cell lysate (eg. Jurkat cell lysate)
- Tissue lysate (eg. Liver tissue)
- Recombinant enzyme
- Purified protein

IV. Kit Contents:

Components	K478-100	Cap Code	Part Number
HPRT Assay Buffer	35 ml	NM	K478-100-1
HPRT Substrate I	1.5 ml	Orange	K478-100-2
HPRT Substrate II	1 vial	Clear	K478-100-3
HPRT Developer	1 vial	Green	K478-100-4
HPRT Enzyme Mix	1 vial	Red	K478-100-5
HPRT Probe	0.4 ml	Blue	K478-100-6
IMP Standard	1 vial	Yellow	K478-100-7
HPRT Positive Control	1 vial	Purple	K478-100-8

V. User Supplied Reagents and Equipment:

- 96-well white plate with flat bottom
- Multi-well spectrophotometer
- Sephadex spin columns (BV# 6564)

VI. Storage Conditions and Reagent Preparation:

Upon arrival, store the kit at -20°C, protected from light. Briefly centrifuge small vials before opening. Read entire protocol before performing the assay.

- **HPRT Assay Buffer:** Warm to room temperature before use.
- **HPRT Substrate I:** Thaw at room temperature before use. Aliquot and store the remaining stock at -20ºC.
- **HPRT Substrate II, HPRT Developer and HPRT Enzyme Mix:** Reconstitute each vial with 220 µl HPRT assay buffer**.** Aliquot and store at -20ºC in the dark. Avoid repeated freeze thaw cycles. Reconstituted developer and enzyme mix are stable for at least 2 months
- **HPRT Probe:** Store at -20°C. Thaw at room temperature before use. *Do not keep on ice***.**
- **IMP Standard:** Reconstitute the vial immediately before first use in 110 µl HPRT Assay Buffer to obtain 100 mM stock. *Dilute IMP immediately before adding to wells for standard curve generation. Keep on ice. Aliquot and store the remaining 100 mM stock at -20º*C. *Do not store diluted solutions.*
- **HPRT Positive Control:** Lyophilized enzyme is stable for 12 months at -20ºC. Reconstitute in 50 µl HPRT buffer. Aliquot and store at -20ºC. Reconstituted enzyme is stable for at least 2 months. *Keep on ice while performing the assay.*

VII. HPRT Activity Assay Protocol:

1. **Sample preparation**: Homogenize cells (4 x 10^5 cells) or tissue (10 mg) with 100 µl ice-cold HPRT Assay buffer to perform lysis and keep on ice for 10 minutes followed by centrifugation at 10,000 x *g* for 15 minutes at 4 ºC. Collect the supernatant (lysate) and estimate protein concentration using preferred method. *We recommend BCA protein assay kit (BV# K813-2500). Protein concentration should range between 0.2 and 1 µg/µl*). Dilute the lysate if needed using HPRT Assay Buffer. Use sephadex spin columns for removal of small molecules that might contribute to a high background. We recommend EZ-Desalt™ Spin Desalting Columns (BV# 6564-25). *Use samples for activity analysis immediately; if that is not possible, they may be stored at -80 ºC for 3-4 days*. Prepare two wells for each

sample labeled "Sample Background Control" (SBC), and "Sample" (S). Add 5 -10 µl sample (1 – 10 µg protein) into each of these wells. For Positive Control, add 5-10 µl of the provided HPRT Positive Control into the desired well. Adjust volume in each well to 50 µl with HPRT Assay Buffer. For Assay Background Control (i.e., substrate background), add 50 µl of HPRT Assay Buffer to a well.

Note: For unknown samples, we suggest testing several concentrations to ensure the readings are within the Standard Curve range.

2. **Standard Curve Generation:** Dilute the reconstituted IMP Standard 1:100 by adding 5 µl of the 0.1 M stock to 495 µl HPRT Assay Buffer to obtain a 1 mM Standard solution. Dilute the 1 mM solution further to obtain 12.5 µM solution by dissolving 2.5 µl of the 1 mM solution in 197.5 µl HPRT Assay buffer   Add 0, 2, 4, 8, 12 and16 µl of the 12.5 µM solution into a series of wells in a white 96-well plate to obtain 25, 50, 100, 150 and 200 pmol/ well. Adjust the volume of each well to 50 µl with HPRT Assay Buffer.

   Note: *Dilute IMP immediate before adding to wells for standard curve generation. Keep on ice.*

3. **Reaction Mix:** Mix enough reagents for the number of assays to be performed. Add IMPSC/SBC Mix to "IMP Standard Curve" wells and "Sample Background Control" wells and Reaction Mix to Assay Background Control (i.e. substrate background), Sample, and Positive Control wells. For each well, prepare 50 µl :

	IMPSC/SBC Mix	Reaction Mix
HPRT Assay Buffer	44 µl	27 µl
HPRT Substrate I	-	15 µl
HPRT Substrate II	-	2 µl
HPRT Developer	2 µl	2 µl
HPRT Enzyme Mix	2 µl	2 µl
HPRT Probe	2 µl	2 µl

   Mix well. Add the reaction mixes to the wells of the 96-well white plate.

   Notes:

   a. Have the plate reader ready at 37°C, at Ex/Em 535/587 nm on kinetic mode set to record fluorescence every 30 seconds.
   b. Prepare reaction mix immediately before adding to wells.

4. **Measurement:** Immediately start recording fluorescence at 30 second intervals for 60 - 90 minutes. Standard curve may be read in either kinetic or end point mode (after 60 minutes).

5. **Calculation:** Subtract the standard background (0 pmol IMP) from IMP standard RFU values, and sample background control RFU values from the sample RFU values respectively. *If assay background control RFU values are higher than sample background control, subtract those values from sample RFU values instead.* Estimate amount of IMP formed using the standard curve. Calculate ΔM, which is the change in amount of IMP between time t_1 and t_2, such that t_1 and t_2 both fall in the linear portion of the reaction. HPRT activity may be calculated using the following equation:

Sample HPRT specific activity = ΔM / (Δt x P) (nmol / (min x µg)) ≡ mUnits / µg ≡ Units / mg

Where: **ΔM =** linear change in IMP concentration during Δt (nmol)
Δt = $t_2 - t_1$ (min)
P = sample protein content added to well (µg)

Unit Definition: One unit of HPRT is the amount of enzyme that produces1 µmol of IMP per minute at pH 8 at 37°C.

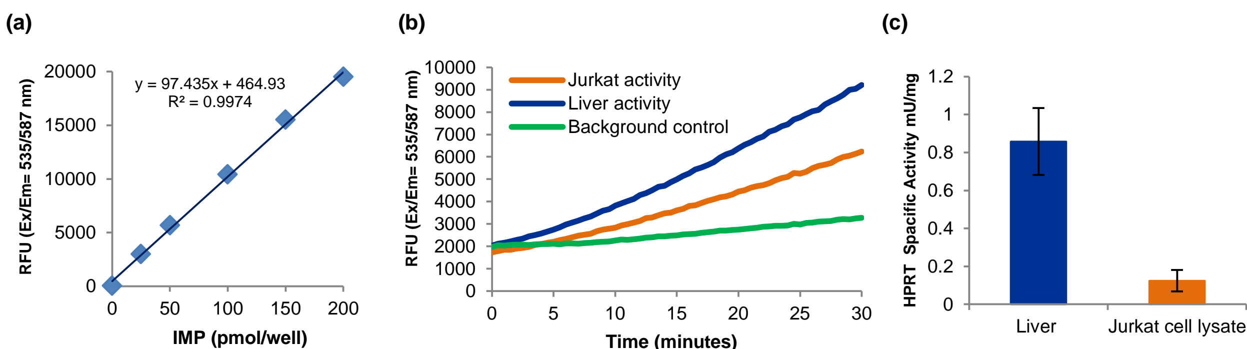


Figure 1: (**a**) IMP standard curve (**b**) Enzyme kinetics for HPRT activity in rat liver lysate (2 µg protein per well) and Jurkat cell lysate (8 µg protein per well) **(c)** HPRT activity in rat liver tissue lysate and jurkat cell lysate.

VIII. RELATED PRODUCTS:

HPRT1, human recombinant (P1092)
Xanthine/Hypoxanthine Colorimetric/Fluorometric Assay Kit (K685)
Inosine Fluorometric Assay Kit (K712)
Purine Nucleoside Phosphorylase Activity Assay Kit (Fluorometric) (K767)
Purine Nucleoside Phosphorylase Activity Assay Kit (Colorimetric) (K768)

FOR RESEARCH USE ONLY! Not to be used on humans.