ab284551 – Hypoxanthine Phosphoribosyl Transferase Activity Assay Kit (Fluorometric)

For the measurement of HPRT activity in biological samples.
For research use only - not intended for diagnostic use.

PLEASE NOTE: With the acquisition of BioVision by Abcam, we have made some changes to component names and packaging to better align with our global standards as we work towards environmental-friendly and efficient growth. You are receiving the same high-quality products as always, with no changes to specifications or protocols.

For overview, typical data and additional information please visit:
http://www.abcam.com/ab284551

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. Hypoxanthine Phosphoribosyl Transferase Activity Assay Kit (ab284551) (previously known as PicoProbe Hypoxanthine Phosphoribosyl Transferase Activity Assay Kit K478) is a simple one-step plate-based assay kit for the measurement of HPRT activity in biological samples. The assay can detect as low as 2 mU HPRT.

Applications

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

Sample Types

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

Storage and Stability

On receipt entire assay kit should be stored at -20°C, protected from light.

Materials Supplied

Item	Quantity	Storage Condition
HPRT Assay Buffer	35 ml	-20°C
HPRT Substrate I	1.5 ml	-20°C
HPRT Substrate II	1 vial	-20°C
HPRT Developer	1 vial	-20°C
Development Enzyme Mix VI/HPRT Enzyme Mix	1 vial	-20°C
PicoProbe I/HPRT Probe	0.4 ml	-20°C
IMP Standard	1 vial	-20°C
HPRT Positive Control	1 vial	-20°C

Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully utilize this assay:

- Multi-well spectrophotometer
- 96-well white plate with flat bottom
- Sephadex spin columns (Cat. No. ab288149)

Reagent Preparation

- Before using the kit, spin the tubes prior to opening.

HPRT Assay Buffer: Warm to room temperature (RT) before use.

HPRT Substrate I: Thaw at room temperature before use. Aliquot and store the remaining stock at -20°C.

HPRT Substrate II, HPRT Developer and Development Enzyme Mix VI/HPRT Enzyme Mix: Reconstitute each vial with 220 µl HPRT assay buffer. Aliquot and store at -20°C in the dark. Avoid repeated freeze thaw cycles. Reconstituted developer and Development Enzyme Mix VI/enzyme mix are stable for at least 2 months.

PicoProbe I/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 -20°C. Do not store diluted solutions.

HPRT Positive Control: Lyophilized enzyme is stable for 12 months at -20°C. Reconstitute in 50 µl HPRT buffer. Aliquot and store at -20°C. Reconstituted enzyme is stable for at least 2 months. Keep on ice while performing the assay.

Assay Protocol

Sample Preparation:

1. 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 °C
2. Collect the supernatant (lysate) and estimate protein concentration using preferred method. We recommend using BCA Protein Assay Kit II (Cat. No. ab287853). Protein concentration should range between 0.2 and 1 µg/µl).
3. 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 Spin Desalting Columns (Cat. No. ab288149)
4. 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.
5. For Positive Control, add 5-10 µl of the provided HPRT Positive Control into the desired well.
6. Adjust volume in each well to 50 µl with HPRT Assay Buffer.
7. For Assay Background Control (i.e., substrate background), add 50 µl of HPRT Assay Buffer to a well.

Δ Notes:

a. *We recommend using the Samples for activity analysis immediately. Otherwise, store the Sample(s) at -80°C for 3-4 days.*

b. *For Unknown Samples, we suggest testing several concentrations to ensure that the readings are within the Standard Curve range*

Standard Curve Generation:

1. 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.
2. 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.
3. 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.

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

Reaction Mix:

1. 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:

Item	IMPSC/SBC Mix	Substrate Mix
HPRT Assay Buffer	44 µl	27 µl
HPRT Substrate I	-	15 µl
HPRT Substrate II	-	2 µl
HPRT Developer	2 µl	2 µl
Development Enzyme Mix VI/HPRT Enzyme Mix	2 µl	2 µl
PicoProbe I/HPRT Probe	2 µl	2 µl

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

Δ 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.

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).

Calculation

1. Subtract the standard background (0 pmol IMP) from IMP standard RFU values, and sample background control RFU values from the sample RFU values respectively.
2. If assay background control RFU values are higher than sample background control, subtract those values from sample RFU values instead.
3. Estimate amount of IMP formed using the standard curve. Calculate ΔM, which is the change in amount of IMP between time t1 and t2, such that t1 and t2 both fall in the linear portion of the reaction. HPRT activity may be calculated using the following equation:

$$\textbf{Sample HPRT specific Activity} = \frac{\Delta M}{(\Delta t \times P)(nmol/(\min \times \mu g))} \equiv \frac{mUnits}{\mu g} \equiv \frac{Units}{mg}$$

Where: **ΔM** = Linear change in IMP concentration during Δt (nmol)
Δt = t2 – t1 (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

Technical Support

For all technical or commercial enquiries please go to:

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