PicoProbe™ Hexokinase Activity Assay Kit (Fluorometric)

(Catalog # K769-100; 100 assays; Store at -20°C)

I. Introduction:

Hexokinase (EC 1.1.1.49; also called HK, 6-Phosphate Glucose Kinase, ATP: D-Hexose 6-Phosphotransferase, ATP-dependent Hexokinase) is found in many organisms including bacteria, plants and mammals. Hexokinases play an important role in glucose metabolism. There are four isoforms (HK-I, II, III and IV). HK-I, HK-II, and HK-III have low Km, while HK-IV (also called Glucokinase) has 100-fold high Km for glucose. Hexokinase deficiency leads to diseases such as X-linked muscular dystrophy and rare autosomal recessive hemolytic anemia. On the other hand, increased hexokinase activity is detected in various human tumors and is associated with metastasis. BioVision's PicoProbe™ Hexokinase Activity Assay kit provides a quick and easy method for monitoring HK activity in a wide variety of samples. In this assay, HK converts glucose into glucose-6-Phosphate, which in turn undergoes a series of reactions and reduce PicoProbe™ to generate intense fluorescent product (Ex/Em = 535/587 nm). The assay is simple, sensitive and high-throughput adaptable and can detect as low as 2 µU of HK activity.

$$\text{Glucose + ATP} \xrightarrow{\text{Hexokinase}} \text{Glucose-6-Phosphate + ADP} \xrightarrow{\text{+ PicoProbe}} \text{Fluorescence (Ex/Em = 535/587 nm)}$$

II. Applications:

- Measurement of Hexokinase activity in various tissues/cells.
- Analysis of Glucose metabolism and Cell signaling in various cell types.
- Screening anti-diabetic drugs.

III. Sample Type:

- Animal tissues: muscle, liver, heart, kidney, etc.
- Cell culture: adherent or suspension cells
- Plant tissues
- Serum

IV. Kit Contents:

Components	K769-100	Cap Code	Part Number
HK Assay Buffer	25 ml	WM	K769-100-1
PicoProbe™ (in DMSO)	0.4 ml	Blue	K769-100-2
HK Substrate	1 ml	White	K769-100-3
ATP	1 Vial	Orange	K769-100-4
HK Enzyme	1 Vial	Green	K769-100-5
HK Developer	1 Vial	Red	K769-100-6
HK Positive Control	1 Vial	Purple	K769-100-7
NADPH Standard (200 nmol)	1 Vial	Yellow	K769-100-8

V. User Supplied Reagents and Equipment:

- 96-well white plate with flat bottom
- Multi-well spectrophotometer (ELISA reader)

VI. Storage Conditions and Reagent Preparation:

Store kit at -20°C, protected from light. Briefly centrifuge small vials prior to opening. Read entire protocol before performing the assay.

- **HK Assay Buffer:** Bring to room temperature before use. Store at 4°C or -20°C.
- **PicoProbe™:** Before use, thaw at room temperature. Store at -20°C. Use within two months.
- **ATP:** Reconstitute with 220 µl dH_2O. Pipette up and down to dissolve completely. Aliquot and store at -20°C. Use within two months.
- **HK Enzyme and HK Developer:** Reconstitute each with 220 µl Assay Buffer. Pipette up and down to dissolve completely. Store at -20°C. Use within two months.
- **HK Positive Control:** Reconstitute with 200 µl Assay Buffer and mix thoroughly. Aliquot and store at -70°C. Avoid freeze/thaw. Use within two months. Keep on ice while in use.
- **NADPH Standard:** Reconstitute with 200 µl dH_2O to generate 1 mM (1 nmol/µl) NADPH Standard solution. Aliquot and store at –20°C. Use within two months. Keep on ice while in use.

VII. HK Activity Assay Protocol:

1. **Sample Preparation:** Homogenize tissue (~10 mg) or cells (1 x 10^6) with 100 µl ice cold HK Assay Buffer. Keep on ice for 10 min. Centrifuge at 10,000 X g, 4°C for 5 min. and collect supernatant. Dilute the supernatant ~10 fold in Assay Buffer and add 1-50 µl into desired well(s) in a 96-well plate. For Positive Control, dilute HK Positive Control 200 times with HK Assay Buffer just before use and add 2-10 µl of diluted HK Positive Control into desired well(s). Adjust the volume of Positive Control and sample wells to 50 µl/well with HK Assay Buffer.

   Notes:

   a. For unknown samples, we suggest doing pilot experiment and testing several amounts of HK to ensure the readings are within the Standard Curve range.

   b. If sample has high background, prepare parallel sample well(s) as sample background control.

   c. Don't store the diluted HK Positive Control.

2. **NADPH Standard Curve:** Dilute NADPH Standard to 40 µM (40 pmol/µl) by adding 40 µl of 1 mM NADPH Standard to 960 µl of dH_2O. Add 0, 2, 4, 6, 8, and 10 µl of 40 µM NADPH Standard into a series of wells in a 96-well plate to generate 0, 80, 160, 240, 320 and 400 pmol/well of NADPH Standard. Adjust the volume to 50 µl/well with HK Assay Buffer.
3. **Reaction Mix:** Mix enough reagents for the number of assays to be performed. For each well, prepare 50 µl Mix containing

	Reaction Mix	*Background Control Mix
HK Assay Buffer	32 µl	44 µl
PicoProbe™	2 µl	2 µl
HK Enzyme	2 µl	2 µl
HK Developer	2 µl	2 µl
ATP	2 µl	----
HK Substrate	10 µl	----

Mix and add 50 µl of Reaction Mix into each well containing Standards, Positive Control, and Samples. Mix well.
* For samples having background, add 50 µl of Background Control Mix to sample background control well(s).

4. **Measurement:** Measure fluorescence (Ex/Em = 535/587 nm) immediately in kinetic mode for 10-40 min. at 25°C.

Note: Incubation time depends on the HK activity in the samples. We recommend measuring fluorescence in kinetic mode, and choosing two time points (T_1 and T_2) in the linear range to calculate the HK activity of the samples. The NADPH Standard Curve can be read in endpoint mode (i.e. at the end of incubation time).

5. **Calculation:** Subtract 0 Standard reading from all readings. Plot the NADPH Standard curve. If sample background control reading is significant, subtract the sample background control reading from sample reading. Calculate the HK activity of the test sample: $\Delta RFU = RFU_2 - RFU_1$. Apply ΔRFU to NADPH Standard Curve to get B pmol of NADPH generated by HK during the reaction time ($\Delta T = T_2 - T_1$).

Sample HK Activity = B/(ΔT X V) x D = pmol/min/µl = µU/µl = mU/ml

Where: **B** is NADPH amount in the sample well from Standard Curve (pmol)
ΔT is reaction time (min.)
V is sample volume added into the reaction well (µl)
D is dilution factor

HK Activity in samples can also be expressed as mU/mg of protein.

Unit Definition: One unit of Hexokinase is the amount of enzyme that generates 1.0 µmol of NADPH per min. at pH 8.0 at 25°C.

(a) (b) (c)

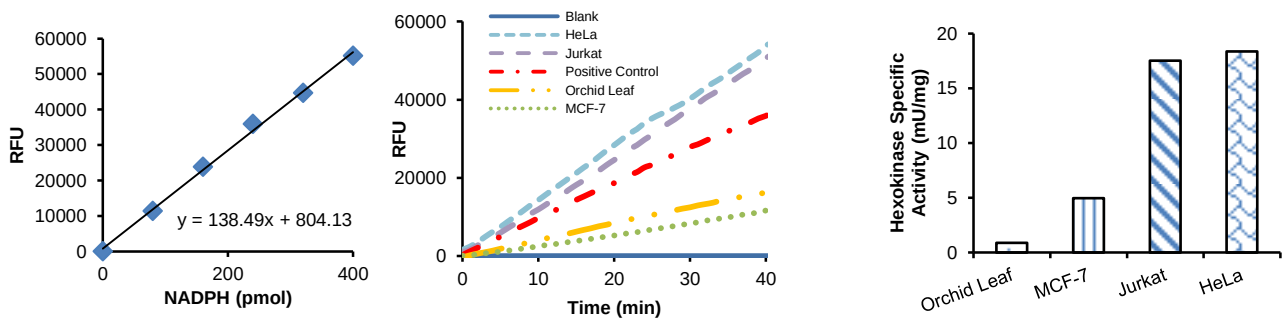

Figure: (a) NADPH Standard Curve. (b) Kinetic measurement of HK Specific Activity in lysates prepared from orchid leaf (4 µg), MCF-7 (0.56 µg), Jurkat (0.65 µg) and HeLa (0.62 µg) cells. (c) HK specific activity of samples mentioned in Fig. b. Assays were performed following the kit protocol.

VIII. RELATED PRODUCTS:

Glucose-6-Phosphate Dehydrogenase Activity Assay Kit (K757)
Pyruvate Colorimetric/Fluorometric Assay Kit (K609)
Triose Phosphate Isomerase Assay Kit (K670)
Lactate Colorimetric Assay Kit II (K627)
Phosphoglucomutase Assay Kit (K774)
PicoProbe™ D-Lactate Fluorometric Assay Kit (K668)
Glucose-6-Phosphate Assay Kit (K657)
Hexokinase Colorimetric Assay Kit (K789)
Pyruvate Dehydrogenase Activity Assay Kit (K679)
PicoProbe™ NADH Assay Kit (K338)
PicoProbe™ NADPH Assay Kit (K349)
Phosphoglucose Isomerase Assay Kit (K775)
Glucose-1-Phosphate Assay Kit (K697)
Glucose Dehydrogenase Activity Assay Kit (K786)

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