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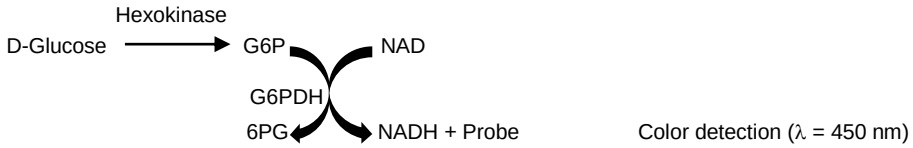
Human Hexokinase II (HKII) Inhibitor Screening Kit (Colorimetric)

(Catalog # K713-100; 100 assays; Store at -20°C)

I. Introduction:

Hexokinases (HK, EC: 2.7.1.1) are found in many organisms including bacteria, plants and mammals and play an important role in glucose metabolism. The Hexokinase family phosphorylates glucose and generates glucose-6-phosphate for glycolysis. Four Hexokinase isoforms (HK-I, II, III and IV) are found in numerous species. HK-I, HK-II and HK-III have low affinity for glucose, one of their natural substrates, while HK-IV show high affinity for this monosaccharide. Recent studies have found increased hexokinase activity in various human metastatic tumors. Moreover, Hexokinase II (HKII) is the main isoform of the Hexokinases and is responsible for malignant phenotypes. HKII binds to the outer mitochondrial membrane via the Voltage-Dependent Anion Channel (VDAC), a Porin-like protein. HKII has become an attractive therapeutic target for its role in cancer metastasis. BioVision's Human Hexokinase II (HKII) Inhibitor Screening kit uses the HKII's ability to convert glucose into glucose-6-phosphate. Glucose-6-phosphate is oxidized by glucose-6-phosphate dehydrogenase to form NADH, which reduces a probe that shows strong absorbance at 450 nm. In the presence of Bromopyruvic Acid, reactions are impeded, thus decreasing the rate and/or extent of generation of Hexokinase -dependent absorbance at OD 450 nm. Hexokinase Inhibitor Control is included to compare the efficacy of test inhibitors. This kit provides a sensitive, quick, and easy method for screening potential inhibitors of Hexokinase. The assay is high-throughput adaptable and can be performed in less than 30 min.



II. Applications:

- Screening/characterizing/studying potential inhibitors of Hexokinase

III. Kit Contents:

Components	K713-100	Cap Code	Part Number
HK Assay Buffer	25 ml	WM	K713-100-1
HK Substrate	1 ml	White	K713-100-2
HK Coenzyme	1 vial	Purple	K713-100-3
HK Converter	1 vial	Green	K713-100-4
HK Developer	1 vial	Red	K713-100-5
Hexokinase II (human)	10 µl	Brown	K713-100-6
HK Inhibitor Control (Bromopyruvic Acid)	1 vial	Orange	K713-100-7

IV. User Supplied Reagents and Equipment:

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

V. 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 -20 °C or 4°C.
- **HK Coenzyme and HK Developer:** Reconstitute each with 220 µl dH_2O. Pipette up and down to dissolve completely. Store at -20°C. Use within two months. Keep on ice while in use.
- **HK Converter:** Reconstitute with 220 µl HK Assay Buffer. Aliquot and store at -20°C. Avoid freeze/thaw. Keep on ice while in use. Use within two months.
- **Hexokinase II:** Ready to use. Store at -20°C.
- **HK Inhibitor Control:** Reconstitute with 100 µl dH_2O. Aliquot and store at -20°C. Avoid freeze/thaw. Keep on ice while in use. Use within two months.

VI. Human Hexokinase II (HKII) Inhibitor Screening Assay Protocol:

1. **Screen Compounds, Inhibitor Control, and Enzyme Control Preparation:** Dissolve candidate inhibitors into an appropriate solvent at highest concentration to be tested. Dilute to 2X desired test concentration with HK Assay Buffer. Add 50 µl diluted candidate inhibitor or HK Assay Buffer into desired wells for Sample Compound [S], and Enzyme Control [EC] (no inhibitor) respectively. For Inhibitor Control [IC], dilute Inhibitor Control 50 times by adding 10 µl Inhibitor Control to 490 µl HK Assay Buffer. Add 50 µl of diluted Inhibitor Control into desired well(s).

   Note:

   Solvents used to solubilize the inhibitors might affect the enzymatic activity. Prepare a solvent control (SC) containing the same final concentration of solvent as in the test well(s) to test the effect of solvent on the enzymatic activity.

2. **Hexokinase Enzyme Preparation:** Make enough Diluted Hexokinase Enzyme for the number of assays to be performed. Dilute Hexokinase 1:100 with assay buffer, e.g. take 2 µl of Hexokinase into 198 µl Assay buffer, mix well. Add 5 µl diluted Hexokinase Enzyme into Sample Compounds [S], Enzyme Control [EC], Solvent Control [SC] and Inhibitor Control [IC] wells. Incubate for 5 min. at 25°C.

Note:
Do not store unused diluted hexokinase. Always prepare a fresh Stock when needed.

3. **Substrate Solution Preparation:** Mix enough reagents for the number of assays to be performed. For each well, prepare 45 µl Substrate Solution Preparation containing:

	Reaction Mix
HK Assay Buffer	29 µl
HK Substrate	10 µl
HK Coenzyme	2 µl
HK Converter	2 µl
HK Developer	2 µl

Mix and add 45 µl of Substrate Solution into each well (Sample Compound, Enzyme Control, and Inhibitor Control). Mix well with gentle shaking.

4. **Measurement:** Measure OD 450nm in kinetic mode for 5-30 min at 25°C. Choose two time points (T_1 & T_2) in the linear range of the plot and obtain the corresponding values for the OD_{450nm} (OD_1 & OD_2).

5. **Calculation:** Calculate the slope for all samples, including Enzyme Control (EC), by dividing the net ΔOD (=OD_2-OD_1) value by the time ΔT (=T_2-T_1). Calculate % relative inhibition as follows. If the values of Solvent Control(s) are significantly different from the Enzyme Control use SC values instead of EC values.

Notes:

a. This is only a primary inhibitor-screening assay and identified candidates have to be validated with independent assay system (We recommend Glucose-6-Phosphate Dehydrogenase Inhibitor Screening Kit (Colorimetric), Catalog #K225).

b. The Relative Activity of the Enzyme Control should be set as 100%.

$$\textbf{Relative Activity } (\%) = \frac{\textbf{Slope of S}}{\textbf{Slope of EC}} \textbf{ X 100}$$

Where: **Slope of EC** is the Enzyme Control Slope
Slope of S is the Sample Compound Slope

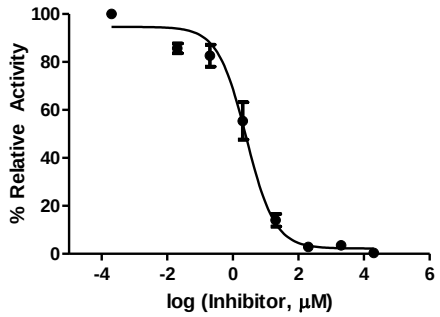


Figure: Inhibition of Human Hexokinase II activity by Bromopyruvic Acid (IC_{50} = 3 µM). Assay was performed following the kit protocol.

VII. RELATED PRODUCTS:

Glucose Assay kit (K606)
Glucose Dehydrogenase Activity Assay Kit (K786)
Glucose-1-Phosphate Colorimetric Assay Kit (K697)
Glucose Uptake Colorimetric Assay Kit (K676)
Glycogen Assay Kit (K646)
Maltose and Glucose Assay Kit (K618)
Glucose and Sucrose Assay Kit (K616)
Glucose-6-Phosphate Dehydrogenase Assay Kit (K757)
PicoProbeTM Glucose-6-Phosphate Assay Kit (K687)
Glucose Uptake Fluorometric Assay Kit (K666)
Hexokinase Colorimetric Assay Kit (K789)
GluTracker™ Glucose Uptake Assay Kit (Cell-Based) (K681)

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