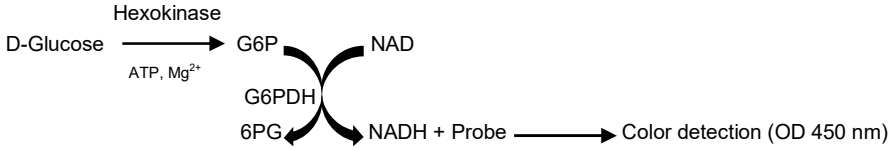
Hexokinase (HK) Inhibitor Screening Kit (Colorimetric) (#BN01045)

(Catalog # BN01045; 100 assays; Store at -20°C)

I. Introduction:

Hexokinases (HK) play an important role in glucose metabolism. Hexokinases phosphorylate glucose and generate glucose-6-phosphate for glycolysis. Hexokinases have four isoforms (HK-I, II, III and IV). HK-I, HK-II and HK-III have low Km, while HK-IV has 100 fold high Km. Hexokinase deficiency leads to severe human diseases such as X-linked muscular dystrophy and a rare autosomal recessive hemolytic anemia. On the other hand, increased hexokinase activity is detected in various tumors and is associated with metastasis. In Assay Genie's Hexokinase Inhibitor Screening kit, glucose is converted to glucose-6-phosphate by hexokinase; the glucose-6-phosphate is oxidized by glucose-6-phosphate dehydrogenase to form NADH, which reduces a colorless probe to a colored product with strong absorbance at 450 nm. In the presence of a Hexokinase inhibitor, the reaction is impeded/abolished resulting in decrease or total loss of absorbance. This assay kit can be used to screen/study/characterize the potential inhibitors of Hexokinase. The assay is simple, high-throughput adaptable and can be performed within 30 min.



II. Application:

- Screening/characterizing/studying potential inhibitors of Hexokinase.

III. Kit Contents:

Components	BN01045	Cap Color	Part Number
HK Assay Buffer	25 ml	WM	BN01045-1
HK Substrate	1 ml	White	BN01045-2
HK Coenzyme	1 vial	Purple	BN01045-3
HK Converter	1 vial	Green	BN01045-4
HK Developer	1 vial	Red	BN01045-5
Hexokinase	1 vial	Purple/White Dot	BN01045-6
HK Inhibitor Control	1 vial	Orange	BN01045-7

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:** Reconstitute with 160 µl HK Assay Buffer. Aliquot and store at -20°C. Avoid freeze/thaw. Keep on ice while in use. Use within two months.
- **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. HK Inhibitor Screening Protocol:

1. **Screen Compounds, Inhibitor Control, and Enzyme Control Preparation:** Dissolve sample inhibitors into an appropriate solvent at the maximum concentration. Dilute to 2X the desired test concentration with HK Assay Buffer. Add 50 µl diluted candidate inhibitor or HK Assay Buffer into desired wells, as Sample [S], or Enzyme Control [EC] (no inhibitor). For Inhibitor Control (IC), dilute Inhibitor Control 5 times by adding 20 µl Inhibitor Control to 80 µl HK Assay Buffer. Add 50 µl of the diluted Inhibitor Control into desired well(s).

   Note: Solvents used to solubilize the inhibitors might affect the enzymatic activity. If solvent effect on enzymatic activity is a concern, prepare a solvent control well with the same final concentration of the solvent as in the inhibitor sample as solvent control.

2. **Hexokinase Enzyme Preparation:** Dilute Hexokinase 1:10 with Assay Buffer. Add 5 µl diluted Hexokinase into Sample, Enzyme Control and Inhibitor Control wells. Incubate for 5 min. at 25°C.

   Note: Discard the diluted hexokinase after use.

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

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. Mix well with gentle shaking.

4. **Measurement:** Measure absorbance (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. **Calculations:** 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).

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

Notes:

a. Irreversible inhibitors that inhibit the Hexokinase activity completely at the tested concentration will have ΔOD = 0 and thus the % Relative Inhibition will be 100%.

b. This is only a primary inhibitor-screening assay and identified candidates have to be validated with independent assay system.

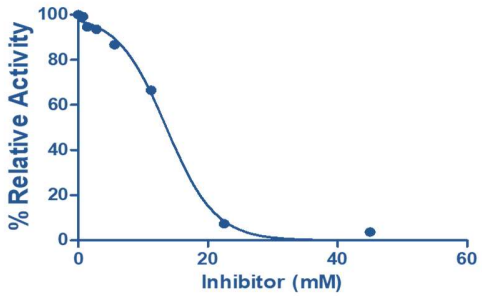


Figure: Inhibition of Hexokinase activity by Hexokinase inhibitor (Bromopyruvic Acid). Assay was performed following the kit protocol.

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