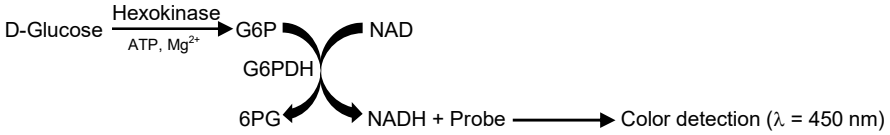
Hexokinase Colorimetric Assay Kit (#BN01002)

(Catalog # BN01002; 100 assays; Store at -20°C)

I. Introduction:

Hexokinases (HK) are found in many organisms including bacteria, plants and mammals and 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 human tumors and is associated with metastasis. Early detection of abnormal hexokinase activity is crucial for diagnosis, prediction and treatment of the disease. In Assay Genie's Hexokinase Assay 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. The assay is simple, sensitive and rapid and can detect hexokinase activity even less than 0.1 mU/well.



II. Application:

- 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:

- Serum
- Animal tissues: Liver, Heart, Kidney etc.
- Cell culture: Adherent or suspension cells.

IV. Kit Contents:

Components	BN01002	Cap Code	Part Number
HK Assay Buffer	25 ml	WM	BN01002-1
HK Substrate	1 ml	White	BN01002-2
HK Coenzyme (Lyophilized)	1 vial	Purple	BN01002-3
HK Enzyme Mix (Lyophilized)	1 vial	Green	BN01002-4
HK Developer (Lyophilized)	1 vial	Red	BN01002-5
NADH Standard (Lyophilized)	1 vial	Yellow	BN01002-6
HK Positive Control (Lyophilized)	1 vial	Orange	BN01002-7

V. User Supplied Reagents and Equipment:

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

VI. Storage and Handling:

Store kit at –20°C, protected from light. Warm all buffers to room temperature before use. Briefly centrifuge all small vials prior to opening.

VII. Reagent Preparation and Storage Conditions:

- **HK Coenzyme:** Reconstitute with 220 µl HK Assay Buffer to generate 0.2 M solution. Store at -20°C. Use within two months. Keep on ice while in use.
- **HK Enzyme Mix:** Reconstitute with 220 µl HK Assay Buffer. Pipette up and down to dissolve completely. Aliquot and store at -20°C. Avoid repeated freeze/thaw cycles. Use within two months. Keep on ice while in use.
- **HK Developer:** Reconstitute with 220 µl dH_2O. Pipette up and down to dissolve completely. Store at -20°C. Use within two months.
- **NADH Standard:** Reconstitute with 400 µl dH_2O to generate 1.25 mM (1.25 nmol/µl) NADH Standard solution. Store at –20°C. Use within two months. Keep on ice while in use.
- **HK Positive Control:** Reconstitute with 100 µl HK Assay Buffer and mix thoroughly. Aliquot and store at –20°C.

VIII. Hexokinase Assay Protocol:

1. **NADH Standard Curve:** Add 0, 2, 4, 6, 8 and 10 µl of 1.25 mM NADH Standard into a series of wells in duplicate in 96 well plate to generate 0, 2.5, 5.0, 7.5, 10 and 12.5 nmol/well of NADH Standard. Adjust volume to 50 µl/well with HK Assay Buffer.
2. **Sample Preparation:** Rapidly homogenize tissue (10 mg) or cells (1 x 10^6) with 200 µl ice cold HK Assay Buffer for 10 minutes on ice. Centrifuge at 12000 rpm for 5 min. Collect the supernatant. Add 1-50 µl sample (40 µg) per well, adjust final volume to 50 µl with HK Assay Buffer. Prepare a parallel sample well as the background control to avoid interference from the NADH in the sample. **Note:** For unknown samples, we suggest testing several doses to ensure the readings are within the standard curve range.
3. **HK Positive Control:** Dilute Positive control solution 1:99 in HK Assay Buffer. Use 1-10 µl of diluted Positive Control into the desired well(s) and adjust the final volume to 50 µl with HK Assay Buffer.

4. **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	34 µl	44 µl
HK Enzyme Mix	2 µl	2 µl
HK Developer	2 µl	2 µl
HK Coenzyme	2 µl	2 µl
HK Substrate	10 µl	----

Add 50 µl of the reaction mix to each well containing the Standard, Positive Control and test samples and 50 µl of background control mix to each well containing the background control sample. Mix well.

5. **Measurement:** Incubate for 20-60 min at room temperature and measure OD_{450nm}. **Note:** Incubation time depends on the Hexokinase activity in the samples. We recommend measuring the OD in a kinetic mode, and choose two time points (T_1 & T_2) in the linear range to calculate the hexokinase activity of the samples. The NADH standard curve can read in endpoint mode (i.e., at the end of incubation time).

6. **Calculation:** Subtract the 0 standard reading from all standard readings. Plot the NADH standard curve. Correct sample background by subtracting the value derived from the background control from all sample readings. Calculate the hexokinase activity of the test sample: $\Delta OD = A_2 - A_1$. Apply the ΔOD to the NADH standard curve to get B nmol of NADH generated by hexokinase during the reaction time ($\Delta T = T_2 - T_1$).

Sample Hexokinase activity = B/(ΔT X V) x Dilution Factor = nmol/min/ml/ = mU/ml

Where: **B** is the NADH amount from standard curve (nmol).
ΔT is the reaction time (min).
V is the sample volume added into the reaction well (ml).

Unit Definition: One unit of hexokinase is the amount of enzyme that will generate 1.0 µmol of NADH per min at pH 8 at room temperature.

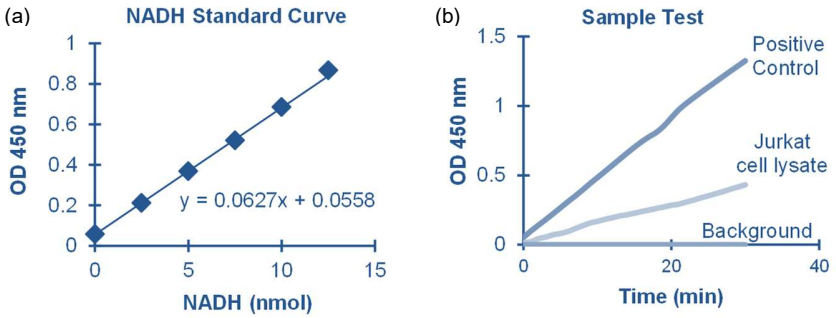


Figure 1: NADH standard curve

(a). Hexokinase activity in Positive Control and Jurkat cell lysate (40 µg)
(b). Assays were performed following kit protocol.

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