Glucokinase Activity Assay Kit (Fluorometric) (#BN01129)

(Catalog # BN01129; 100 assays; Store at -20°C)

I. Introduction:

Glucokinase (also called GCK, hexokinase type IV or D and ATP: D-hexose 6-phosphotransferease; EC 2.7.1.1) is expressed in specific types of tissues: liver, pancreas, small intestine and brain. Glucokinase functions as a glucose sensor, triggering shifts in carbohydrate metabolism or cell function in response to the levels of glucose in blood, such as nutritional and hormonal molecular pathways. Unlike other Hexokinases, Glucokinase has a relatively low affinity for glucose and it is not inhibited by physiological concentrations of glucose 6-phosphate. Mutations in the gene encoding GCK can cause both hyperglycemia and hypoglycemia. Due to the major role of Glucokinase in controlling blood glucose homeostasis, Glucokinase is currently considered as a strong candidate target for the treatment of Hyperglycemia, a condition encountered in Type 2 Diabetic patients. Assay Genie's Glucokinase Activity Assay Kit provides a quick and easy method for monitoring GCK activity in wide variety of samples. In this assay, GCK converts glucose into glucose-6-phosphate, which in turn is converted into a series of intermediates that reduce generating an intense fluorescence product (Ex/Em=535/587nm). The assay is simple, specific, sensitive and high-throughput adaptable and can detect as low as 2 µU of GCK activity.

II. Applications:

- Measurement of Glucokinase activity in various tissues/cells.
- Analysis of Glucose metabolism in various cell types

III. Sample Type:

- Tissue Homogenates: Liver tissue
- Cell Lysates: Hep G2 Cell Lysates

IV. Kit Contents:

Components	BN01129	Cap Code	Part Number
GCK Assay Buffer	25 ml	WM	BN01129-1
GenieProbe (in DMSO)	0.4 ml	Blue	BN01129-2
DTT (1M)	1 ml	Green/white Dot	BN01129-3
GCK Substrate	1 ml	Blue	BN01129-4
Sample Background Reagent	1 ml	Brown	BN01129-5
ATP (Lyophilized)	1 vial	Orange	BN01129-6
GCK Enzyme Mix (Lyophilized)	1 vial	Green	BN01129-7
GCK Developer (Lyophilized)	1 vial	Red	BN01129-8
GCK Pos Control (Lyophilized)	1 vial	Violet	BN01129-9
NADPH Standard (200 nmol) (Lyophilized)	1 vial	Yellow	BN01129-10

V. User Supplied Reagents and Equipment:

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

VI. Storage Conditions and Reagent Preparation:

Store kit at -20°C, protect from light. Briefly centrifuge small vials prior to opening. Read entire protocol before performing the assay. Upon opening, use within two months.

- **GCK Assay Buffer:** Store at either 4 ºC or -20 ºC. Bring to room temperature before use.
- **GenieProbe:** Before use, thaw at room temperature. Store at -20°C.
- **ATP:** Reconstitute with 440 µl dH_2O. Pipette up and down to dissolve completely. Aliquot and store at -20°C.
- **GCK Enzyme Mix and GCK Developer:** Reconstitute each vial with 440 µl GCK Assay Buffer. Pipette up and down to dissolve completely. Store at -20 ºC.
- **GCK Positive Control:** Reconstitute with 20 µl **GCK Assay Buffer containing 2.5 mM DTT** (dilute 2 µl of 1 M DTT with 798 µl of GCK Assay Buffer, use 20 µl of this buffer) and mix thoroughly. Aliquot and **store at -80 ºC**. Avoid freeze/thaw. Keep on ice. while in use.
- **NADPH Standard:** Reconstitute with 200 µl GCK Assay Buffer to generate 1 mM (1 nmol/µl) NADPH Standard Solution. Aliquot and store at -20 °C. Keep on ice while in use.

VII. Glucokinase Activity Assay Protocol:

1. **Sample Preparation:** Homogenize tissue (100 mg) or pelleted cells (~1 x 10^6) with 500 µl ice-cold **GCK Assay Buffer containing 2.5 mM DTT** and keep on ice for 10 min. Centrifuge samples at 12,000 x g at 4 ºC for 10 min. and collect the supernatant. For Sample wells: Dilute the supernatant 10-20 fold in **GCK Assay Buffer** and add 2-10 µl of diluted samples into well(s) of a 96-well clear plate. For Sample background control: Prepare parallel well(s) with same volume(s) of diluted samples. For Positive Control, dilute reconstituted GCK Positive Control 20-fold with **GCK Assay Buffer** prior experiment and add 2-10 µl of **diluted** GCK Positive Control into desired wells(s). Adjust the volume of Positive Control, Sample wells, and Sample Background Control to 50 µl/well with GCK Assay Buffer.

Note:

a. High concentrations of DTT would generate non-specific signal on Reagent Background and Sample Background. We recommend to dilute Samples and GCK Positive Control 10-20 fold with GCK Assay Buffer not supplemented with DTT.

b. For unknown samples, we recommend doing pilot experiment and testing several doses to ensure the readings are within the Standard Curve range and the signal kinetics are within the linear range.

c. Do not store diluted GCK Positive Control.

2. **Standard Curve Preparation:** Dilute NADPH Standard to 100 µM (100 pmol/µl) by adding 10 µl of 1 mM NADPH Standard to 90 µl of GSK Assay Buffer. Add 0, 2, 4, 6, 8, and 10 µl of 100 µM NADPH Standard into a series of wells in a 96-well clear plate to generate 0, 200, 400, 600, 800, 1000 pmol/well of NADPH Standard. Adjust the volume to 50 µl/well with GCK Assay Buffer.
3. **Reaction Mix Preparation:** Mix enough reagents for the number of assays to be performed. For each well, prepare 50 µl Mix containing:

	Reaction Mix	Sample Background Mix
GCK Assay Buffer	30 µl	30 µl
GenieProbe	4 µl	4 µl
GCK Enzyme	2 µl	2 µl
GCK Developer	2 µl	2 µl
ATP	2 µl	2 µl
GCK Substrate	10 µl	---
Sample Background Reagent	---	10 µl

Mix and add 50 µl of the Reaction Mix to well(s) containing Positive Control, Standards and Sample(s). Add 50 µl of the Background Mix to well(s) containing Sample Background Control.

4. **Measurement:** Measure fluorescence (Ex/Em=535/587 nm) in kinetic mode for 20- 30 min at room temperature.
Note: Incubation time depends on the GCK 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 GCK 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 Standard readings. Plot the NADPH Standard Curve and obtain the slope of the curve (**ΔRFU/pmol**); Calculate the background-corrected sample ΔRFU ($\Delta RFU=RFU_2-RFU_1$) by subtracting Sample Background Control ΔRFU from Sample ΔRFU and apply to NADPH Standard Curve to obtain the corresponding amount of NADPH formed (**B**, pmol) during the reading time ($\Delta t=t_2-t_1$). Calculate the GCK activity of the test samples:

Sample GCK Activity = B/ (Δt * V * P) x D = pmol/min/µg = mU/mg

Where: **B** = NADPH amount from Standard Curve (pmol)
Δt = Reaction time (min.)
V = Sample volume added into the reaction well (µl)
P = Sample Concentration in µg-protein/µl
D = Sample Dilution Factor

Unit Definition: One unit of Glucokinase activity is the amount of enzyme that catalyzes the release of 1.0 µmol of NADPH per min. at pH 8.0 and room temperature.

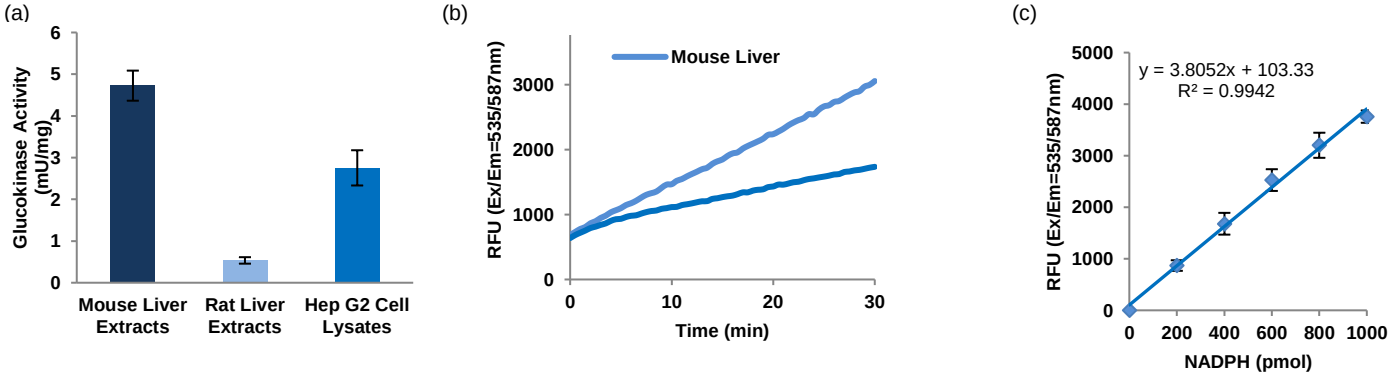

Figure: (a) NADPH Standard Curve. **(b)** GCK Activity in Mouse Liver. **(c)** Measurement of GCK activity in Mouse Liver tissue extracts (2 µg protein); Rat Liver tissue extracts (5 µg protein) and Hep G2 Cell Lysates (2 µg protein). All assays were performed following kit protocols.

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