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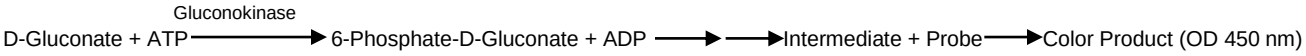
Gluconokinase Activity Assay Kit (Colorimetric)

(Catalog # K319-100; 100 assays; Store at -20°C)

I. Introduction:

Gluconokinase (ATP:D-gluconate 6-phosphotransferase or Gluconate Kinase; EC:2.7.1.12) is a key enzyme for Gluconate degradation pathway. In *E. coli* and yeast, Gluconokinase can convert gluconate into 6-Phosphate-D-Gluconate in an ATP dependent manner. Through Hexose Monophosphate Shunt (HMS) pathway, 6-Phosphate-D-Gluconate generates ribose-6-phosphate, which is critical for nucleotides and nucleic acid synthesis. Little is known of the mechanism of gluconate metabolism in humans despite its widespread use in medicine and consumer products. BioVision's Gluconokinase Assay kit provides a quick and easy way for monitoring Gluconokinase activity in a variety of samples. In this kit, Gluconokinase converts Gluconate into 6-Phosphate-D-Gluconate in an ATP dependent manner. 6-Phosphate-D-Gluconate and ADP in turn undergoe a series of reactions to form an intermediate, which reacts with the probe to form a colored product with strong absorbance (OD 450 nm). The assay is simple, sensitive, and high-throughput adaptable. Detection limit: < 0.1mU.



II. Application:

- Measurement of Gluconokinase activity in various samples
- Mechanistic study of Pentose Phosphate Pathway

III. Sample Type:

- Prokaryote such as: *E.coli*
- Animal tissues such as liver, kidney, etc.
- Adherent or suspension cells.

IV. Kit Contents:

Components	K319-100	Cap Code	Part Number
Gluconokinase Assay Buffer	25 ml	WM	K319-100-1
Gluconokinase Substrate	1 Vial	Blue	K319-100-2
ATP	1 Vial	Orange	K319-100-3
Gluconokinase Converting Enzyme	1 Vial	Purple	K319-100-4
Gluconokinase Developer	1 Vial	Green	K319-100-5
Gluconokinase Probe	1 Vial	Red	K319-100-6
NADH Standard	1 Vial	Yellow	K319-100-7
Gluconokinase Positive Control	1 Vial	Brown	K319-100-8

V. User Supplied Reagents and Equipment:

- 96-well clear 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.

- **Gluconokinase Assay Buffer:** Warm to room temperature before use. Store at -20°C.
- **Gluconokinase Substrate, ATP and Gluconokinase Probe:** Reconstitute with 220 µl dH_2O. Pipette up and down to dissolve completely. Store at -20°C. Use within two months.
- **Gluconokinase Converting Enzyme and Gluconokinase Developer:** Reconstitute with 220 µl Assay Buffer. 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 NADH Standard. Pipette up and down to dissolve completely. Store at -20°C. Use within two months.
- **Gluconokinase Positive Control:** Reconstitute with 200 µl Gluconokinase Assay Buffer and mix thoroughly. Aliquot and store at -20°C. Keep on ice while in use. Use within two months.

VII. Gluconokinase Activity Assay Protocol:

1. **Sample Preparation:** Rapidly homogenize tissue (5 mg) or cells (1 x 10^6) with 100 µl ice cold Gluconokinase Assay Buffer. Keep on ice for 10 min. Centrifuge at 10,000 X g, 4°C for 5 min. and collect supernatant. Add 10-50 µl supernatant per well and adjust the volume to 50 µl/well with Gluconokinase Assay Buffer.

   For Gluconokinase Positive Control, add 1-10 µl of Gluconokinase Positive Control into desired well(s) and adjust the volume to 50 µl/well with Gluconokinase Assay Buffer.

   Notes:

   a. For unknown samples, we suggest doing pilot experiment & testing several doses to ensure the readings are within the Standard Curve range.

   b. For samples having background, prepare parallel sample well(s) as sample background control(s).

c. The small molecules in some tissue samples such as liver may interfere with assay. To remove the small molecules, we suggest removing these molecules from sample by using 10 kDa Spin Column (Cat. 1997) before performing the assay.

2. Standard Curve: Add 0, 2, 4, 6, 8 and 10 µl of 1.25 mM NADH Standard into a series of wells in a 96-well plate to generate 0, 2.5, 5, 7.5, 10 and 12.5 nmol/well of NADH Standard. Adjust the volume to 50 µl with Gluconokinase Assay Buffer.

3. Reaction Mix: Mix enough reagents for the number of assays to be performed. For each well, prepare 50 µl Reaction Mix containing:

	Reaction Mix	Background Control Mix*
Gluconokinase Assay Buffer	40 µl	44 µl
Gluconokinase Substrate	2 µl	----
ATP	2 µl	----
Gluconokinase Converting Enzyme	2 µl	2 µl
Gluconokinase Developer	2 µl	2 µl
Gluconokinase Probe	2 µl	2 µl

Mix well and add 50 µl of Reaction Mix to each well containing Standard, Positive Control and samples.

Note: *Add 50 µl of Background Control Mix to background control samples.

4. Measurement: Measure absorbance (450 nm) immediately in kinetic mode for 5-30 min. at 37°C.

Note: Incubation time depends on the Gluconokinase activity in the samples. We recommend measuring OD in kinetic mode, and choosing two time points (T_1 & T_2) in the linear range to calculate the Gluconokinase activity of the samples. The NADH 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 NADH Standard Curve. If sample background control reading is significant, subtract sample background control reading from sample reading.

Calculate the Gluconokinase activity of the test sample: $\Delta OD = A_2 - A_1$. Apply ΔOD to the NADH Standard Curve to get B nmol of NADH generated by Gluconokinase during the reaction time ($\Delta T = T_2 - T_1$).

Sample Gluconokinase Activity = B/(T X V) x Dilution Factor = nmol/min/µl = mU/µl = U/ml

Where: **B** is NADH amount in the sample well from Standard Curve (nmol).
T is reaction time (min.).
V is sample volume added into the reaction well (µl).

Unit Definition: One unit of Gluconokinase is the amount of enzyme that generates 1.0 µmol of NADH per min. at pH7.4 at 37°C.

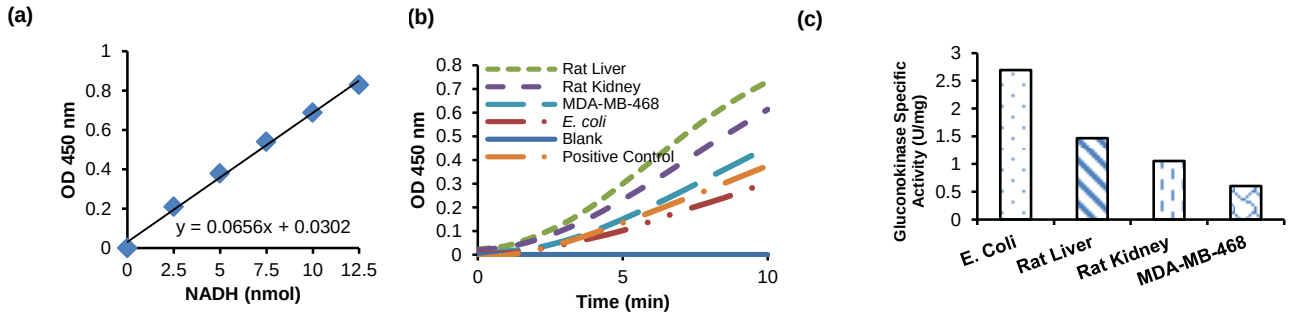


Figure: (a) NADH Standard Curve. (b) Measurement of Gluconokinase activity in Positive Control (1 µl) & lysates from rat liver (1 µg), rat kidney (1 µg), MDA-MB-468 (1 µg) and *E. coli* (0.1 µg). (c) Gluconokinase specific activity in the samples mentioned in figure b. Assay was performed following the kit protocol.

VIII. RELATED PRODUCTS:

Gluconate Colorimetric Assay Kit (K683)
Hexokinase Colorimetric Assay Kit (K789)
Glucose-6-Phosphate Dehydrogenase Activity Assay Kit (K757)
Fumarase Activity Assay Kit (K596)
Pyruvate Colorimetric/Fluorometric Assay Kit (K609)
Succinate Dehydrogenase Activity Assay Kit (K660)
Phosphofructokinase Activity Assay Kit (K776)
Glucose Colorimetric/Fluorometric Assay Kit (K606)
Glucose Dehydrogenase Activity Assay Kit (K786)
Phosphoglucose Isomerase Activity Assay Kit (K775)
Malate Dehydrogenase Activity Assay Kit (K654)
Triose Phosphate Isomerase Activity Assay Kit (K670)
Succinyl-CoA Synthetase Activity Assay Kit (K597)
Aldolase Activity Assay Kit (K665)

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