Version 2b, Last updated 15 March 2024

ab273303 Glucokinase Activity Assay Kit (Fluorometric)

View Kit datasheet: https://www.abcam.com/ab273303
(use https://www.abcam.cn/ab273303 for china, or
https://www.abcam.co.jp/ab273303 for Japan)

For the determination of Glucokinase activity in tissue and cell samples.

This product is for research use only and is not intended for diagnostic use.

PLEASE NOTE: With the acquisition of BioVision by Abcam, we have made some changes to component names and packaging to better align with our global standards as we work towards environmental-friendly and efficient growth. You are receiving the same high-quality products as always, with no changes to specifications or protocols.

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1. Overview

Glucokinase (GCK) Activity Assay Kit (Fluorometric) (ab273303) 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 the PicoProbe I/Probe 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.

2. Protocol Summary

Prepare all reagents as directed.

Prepare Samples, Background Control and Positive Control as directed.

Prepare standards.

Add Positive Control, Samples, Background Control to appropriate wells and adjust volume to 50 µL.

Add Reaction Mix or Sample Background Mix (50 µL).

Measure fluorescence (Ex/Em= 535/587 nm) in a kinetic mode for 20 - 30 minutes at room temperature .

Calculate GCK activity using equation.

3. Precautions

Please read these instructions carefully prior to beginning the assay.

- All kit components have been formulated and quality control tested to function successfully as a kit.
- We understand that, occasionally, experimental protocols might need to be modified to meet unique experimental circumstances. However, we cannot guarantee the performance of the product outside the conditions detailed in this protocol booklet.
- Reagents should be treated as possible mutagens and should be handled with care and disposed of properly. Please review the Safety Datasheet (SDS) provided with the product for information on the specific components.
- Observe good laboratory practices. Gloves, lab coat, and protective eyewear should always be worn. Never pipette by mouth. Do not eat, drink or smoke in the laboratory areas.
- All biological materials should be treated as potentially hazardous and handled as such. They should be disposed of in accordance with established safety procedures.

4. Storage and Stability

Store kit at -20°C in the dark immediately upon receipt. Kit has a storage time of 2 months from receipt.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in the Reagent Preparation section.

Aliquot components in working volumes before storing at the recommended temperature.

5. Limitations

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

6. Materials Supplied

Item	Quantity	Storage temperature
GCK Assay Buffer	25 mL	-20°C
PicoProbe I/Probe (in DMSO)	0.4 mL	-20°C
DTT I/DTT (1 M)	1 mL	-20°C
Glucose Substrate/GCK Substrate	1 mL	-20°C
Sample Background Reagent	1 mL	-20°C
ATP/ATP (Lyophilized)	1 vial	-20°C
Development Enzyme Mix IX/GCK Enzyme Mix (Lyophilized)	1 vial	-20°C
GCK Developer Mix/GCK Developer (Lyophilized)	1 vial	-20°C
GCK Positive Control/GCK Positive Control (Lyophilized)	1 vial	-20°C
NADPH Standard/NADPH Standard (200 nmol) (Lyophilized)	1 vial	-20°C

7. Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully perform this assay:

- Fluorescence microplate reader capable of measuring fluorescence at Ex/Em = 535/587 nm
- 96-well black plate with flat bottom
- Dounce Tissue Homogenizer

8. Technical Hints

- **This kit is sold based on number of tests. A "test" simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.**
- Selected components in this kit are supplied in surplus amount to account for additional dilutions, evaporation, or instrumentation settings where higher volumes are required. They should be disposed of in accordance with established safety procedures.
- Avoid foaming or bubbles when mixing or reconstituting components.
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions.
- Ensure plates are properly sealed or covered during incubation steps.
- Ensure all reagents and solutions are at the appropriate temperature before starting the assay.
- Samples generating values that are greater than the most concentrated standard should be further diluted in the appropriate sample dilution buffer.
- Make sure all necessary equipment is switched on and set at the appropriate temperature.

9. Reagent Preparation

- Briefly centrifuge small vials at low speed prior to opening.
- Upon opening, use within two months.

9.1 **GCK Assay Buffer:**

Ready to use. Bring to room temperature before use. Store at 4 °C or -20 °C.

9.2 **PicoProbe I/Probe (in DMSO):**

Ready to use. Thaw before use. Store at -20 °C.

9.3 **DTT I/DTT (1 M):**

Dilute 2 µl of DTT I/1 M DTT with 798 µl of GCK Assay Buffer to create GCK Assay Buffer containing 2.5 mM DTT I/DTT .

9.4 **Glucose Substrate/GCK Substrate:**

Ready to use. Store at -20 °C.

9.5 **Sample Background Reagent:**

Ready to use. Store at -20 °C.

9.6 **ATP/ATP (Lyophilized):**

Reconstitute with 440 µl dH_2O. Pipette up and down to dissolve completely. Divide into aliquots and store at -20°C.

9.7 **Development Enzyme Mix IX/GCK Enzyme Mix (Lyophilized):**

Reconstitute with 440 µl GCK Assay Buffer. Pipette up and down to dissolve completely.
Store at -20 °C.

9.8 **GCK Developer Mix/GCK Developer (Lyophilized):**

Reconstitute with 440 µl GCK Assay Buffer. Pipette up and down to dissolve completely. Store at -20 °C.

9.9 **GCK Positive Control/GCK Positive Control (Lyophilized):**

Reconstitute with 20 µl 2.5 mM DTT I/DTT in GCK Assay Buffer (prepared in step 9.3) and mix thoroughly. Keep on ice while in use. Divide into aliquots and store at -80 °C. Avoid freeze/thaw.

9.10 **NADPH Standard/NADPH Standard (200 nmol) (Lyophilized):**

Reconstitute with 200 µl GCK Assay Buffer to generate 1 mM (1 nmol/µl) NADPH Standard Solution. Keep on ice while in use. Aliquot and store at -20 °C.

10. Sample Preparation

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

Tissue and cell preparation:

10.1 Homogenize tissue (100 mg) or pelleted cells (~1 x 10^6) with 500 µl ice-cold 2.5 mM DTT I/DTT in GCK Assay Buffer (prepared in step 9.3).
10.2 Keep on ice for 10 mins.
10.3 Centrifuge samples at 12,000 *x g* at 4 °C for 10 mins.
10.4 Collect the supernatant.
10.5 Dilute the supernatant 10-20 fold in GCK Assay Buffer prior experiment.

Positive Control:

10.6 Dilute reconstituted GSK Positive Control 10-fold with GCK Assay Buffer prior experiment.

Δ Note: Do not store diluted GCK Positive Control.

11. Standard Curve

Thaw all reagents thoroughly and mix gently.

11.1 Dilute NADPH Standard to 50 µM (50 pmol/µL) by adding 5 µL of 1 mM NADPH Standard to 95 µL of GSK Assay Buffer.

11.2 Add 0, 2, 4, 6, 8, and 10 µL of 50 µM NADPH Standard into a series of wells in a 96-well black plate.

11.3 Adjust the volume to 50 µl/well with GCK Assay Buffer. This will generate 0, 100, 200, 300, 400, 500 pmol/well of NADPH Standard, respectively.

Standard #	50 µM NADPH Standard (µL)	GCK Assay Buffer (µL)	NADPH (pmol/well)
1	0	50	0
2	2	48	100
3	4	46	200
4	6	44	300
5	8	42	400
6	10	40	500

12. Assay Procedure

– Thaw all reagents thoroughly and mix gently.

12.1 Add 10 µl of diluted samples into Sample and Sample Background Control wells of a 96-well black plate.

12.2 For Positive Control, add 10 µl of diluted GCK Positive Control into desired wells.

12.3 Adjust the volume of Positive Control, Sample, and Sample Background Control wells to 50 µl/well with GCK Assay Buffer.

Reaction Mix:

12.4 Prepare enough reagents for the number of assays to be performed. For each well, prepare 50 µl of appropriate Mix:

Component	Reaction (µL)	Sample Background (µL)
GCK Assay Buffer	30	30
PicoProbe I/Probe	4	4
GCK Enzyme	2	2
GCK Developer Mix/GCK Developer	2	2
ATP	2	2
Glucose Substrate/GCK Substrate	10	---
Sample Background Reagent	---	10

12.5 Mix and add 50 µl of the Reaction Mix to wells containing Positive Control, Standards and Sample(s).

12.6 Add 50 µl of the Background Mix to wells containing Sample Background Control.

12.7 Measure fluorescence (Ex/Em 535/587 nm) in a kinetic mode for 20 - 30 minutes at room temperature.

Δ 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).

13. Calculations

13.1 Subtract 0 Standard reading from all Standard readings.
13.2 Plot the NADPH Standard Curve and obtain the slope of the curve (ΔRFU/pmol).
13.3 Calculate the background-corrected sample ΔRFU ($\Delta RFU=RFU_2-RFU_1$) by subtracting Sample Background Control ΔRFU from Sample ΔRFU.
13.4 Apply to NADPH Standard Curve to obtain the corresponding amount of NADPH formed (B, pmol) during the reading time ($\Delta t=t_2-t_1$).
13.5 Calculate the GCK activity of the test samples:

$$Sample\ GCK\ activity\ = \frac{B}{(\Delta t\ x\ V\ x\ P)} x\ D \quad (pmol/min/\mu g)$$

B = NADPH amount from Standard Curve (pmol)
Δt = Reaction time (mins)
V = Sample volume added into the reaction well (µl)
P = Sample Concentration in µg-protein/µl
D = Dilution factor (D = 1 for undiluted Samples)

Unit definition:

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

14. Typical Data

Typical standard curve – data provided **for demonstration purposes only**. A new standard curve must be generated for each assay performed.

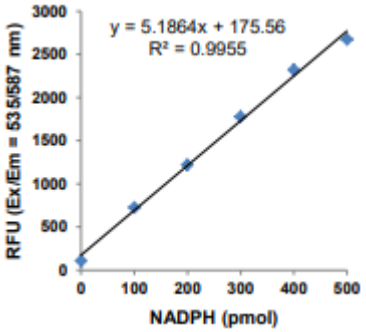

Figure 1. NADPH Standard Curve.

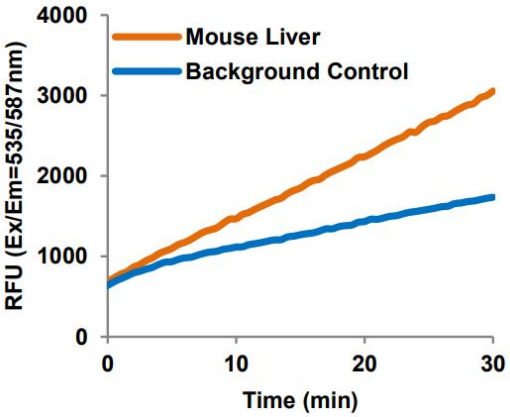

Figure 2. GCK activity in mouse liver.

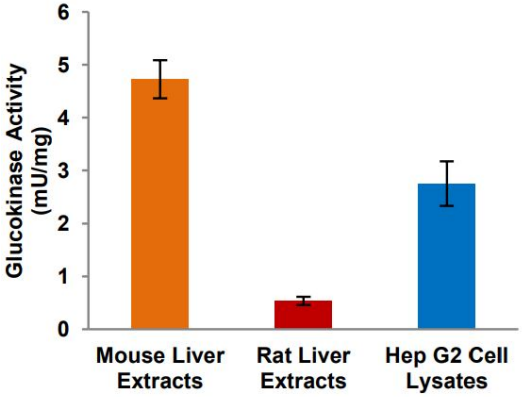

Figure 3. Measurement of GCK activity in mouse liver tissue extracts (2 µg protein); rat liver tissue extracts (5 µg protein) and HepG2 cell lysates (2 µg protein).

15. FAQ / Troubleshooting

General troubleshooting points are found at www.abcam.com/assaykitguidelines.

16. Notes

Technical Support

For all technical or commercial enquiries please go to:

www.abcam.com/contactus

www.abcam.cn/contactus (China)

www.abcam.co.jp/contactus (Japan)