Glycerol Kinase (GK) Assay Kit

Catalog No.: abx294448

Size: 96 tests

Detection Range: 9.28 U/L – 170 U/L

Sensitivity: 9.28 U/L

Storage: Store all components at -20°C in the dark.

Application: For detection and quantification of Glycerol Kinase activity in animal tissue homogenates.

Introduction

Glycerol Kinase (GK) functions as the rate-limiting enzyme in glycerol metabolism, and its deficiency directly impairs the cellular ability to utilize glycerol.

Abbexa's Glycerol Kinase (GK) Assay Kit is a quick, convenient, and sensitive method for measuring and calculating Glycerol Kinase activity. Glycerol Kinase catalyzes the phosphorylation of glycerol which produces glycerol-3-phosphate. Glycerophosphate oxidase oxidizes the product to produce dihydroxyacetone phosphate and hydrogen peroxide. The hydrogen peroxide that is produced then reacts with the chromogenic reagent to produce a compound with an absorbance maximum at 555 nm. The change in intensity of the color is proportional to the Glycerol Kinase activity, which can then be calculated.

Kit components

1. 96-well microplate
2. Detection Reagent: 12 ml
3. Standard: 1 vial
4. Stop Solution: 12 ml
5. Plate sealer: 2

Materials required but not provided

1. Microplate reader (555 nm)
2. Double-distilled water
3. Normal saline (0.9 % NaCl)
4. PBS (0.01 M, pH 7.4)
5. Pipette and pipette tips
6. 1.5 ml microcentrifuge tubes
7. Centrifuge
8. Vortex mixer
9. Incubator

For Reference Only

Protocol

A. Preparation of samples and reagents

1. Samples

Isolate the test samples soon after collecting and analyze immediately or aliquot and store at -20°C for long-term storage. Avoid multiple freeze-thaw cycles.

The following sample preparation method is intended as a guide and may be adjusted as required depending on the specific samples used.

- **Tissue Homogenates:** Carefully weigh at least 0.02 g of tissue and wash with cold PBS (0.01 M, pH 7.4). For each 20 mg of tissue, add into 180 µl normal saline (0.9 % NaCl). Homogenize manually, using a mechanical homogenizer in an ice water bath at 4°C. Centrifuge at 10,000 × g for 10 minutes, then carefully collect the supernatant. Assay immediately and keep on ice for detection.

  Note: To calculate Glycerol Kinase activity in tissue homogenates using the formula in section **C. Calculation of Results**, the total protein concentration of the supernatant must be determined separately (**abx097193**).

It is recommended to carry out a preliminary experiment to determine the optimal dilution factor of samples before carrying out the formal experiment. Dilute samples into different concentrations with normal saline (0.9 % NaCl), then carry out the assay procedure. The recommended dilution factor for different samples is as follows (for reference only):

Sample Type	Dilution Factor
10 % Mouse liver tissue homogenate	1
10 % Mouse kidney tissue homogenate	1
10 % Mouse small intestine tissue homogenate	1

Note:

- Fresh samples or recently obtained samples are recommended to prevent degradation and denaturalization that may lead to erroneous results.
- Lysis buffers may interfere with the kit. It is therefore recommended to use mechanical lysis methods for tissue homogenates.

2. Reagents

- **Detection Reagent:** Unused Detection Reagent can be aliquoted and stored at -20°C for up to 6 months in the dark.
- **4.25 mmol/L Standard Solution:** Dissolve one vial of Standard in 5 ml of double-distilled water. Mix thoroughly to ensure it has fully dissolved. The solution can be stored at -20°C for up to one month.
- **Standards:** Label 7 tubes with 4.25 mmol/L, 3.40 mmol/L, 2.98 mmol/L, 2.13 mmol/L, 1.70 mmol/L, 1.28 mmol/L, and 0.85 mmol/L. Add 200 µl, 160 µl, 140 µl, 100 µl, 80 µl, 60 µl, and 40 µl of 4.25 mmol/L Standard Solution to the 4.25 mmol/L, 3.40 mmol/L, 2.98 mmol/L, 2.13 mmol/L, 1.70 mmol/L, 1.28 mmol/L, and 0.85 mmol/L tubes respectively, followed by 0 µl, 40 µl, 60 µl, 100 µl, 120 µl, 140 µl, and 160 µl of double-distilled water, to prepare Standard Dilutions with concentrations 4.25 mmol/L, 3.40 mmol/L, 2.98 mmol/L, 2.13 mmol/L, 1.70 mmol/L, 1.28 mmol/L, and 0.85 mmol/L. These volumes are summarized in the following table:

For Reference Only

Standard Dilution (mmol/L)	4.25	3.40	2.98	2.13	1.70	1.28	0.85
4.25 mmol/L Standard Solution (µl)	200	160	140	100	80	60	40
Double-distilled water (µl)	0	40	60	100	120	140	160

For the blank, or 0 mmol/L standard, use pure double-distilled water. The volume of each standard will be 200 µl.

Note:

- Allow all reagents to equilibrate to room temperature before use.

For Reference Only

B. Assay Procedure

Pre-heat the incubator and ensure it has reached a stable temperature before use.

1. Assign and record microplate well positions for each sample, standard and blank. *It is strongly recommended to test all wells in duplicate*.
2. Add 10 µl of each standard dilution to the corresponding standard wells.
3. Add 10 µl double-distilled water to the blank wells.
4. Add 10 µl of sample to the sample wells.
5. Add 100 µl of Detection Reagent to all wells.
6. Mix thoroughly for 5 seconds using a microplate shaker or by tapping the plate gently, then measure the OD of the sample wells at 555 nm using a microplate reader. Record these values as $A_{1\ Sample}$.
7. Cover the plate with a plate sealer, then incubate at 37°C for 25 minutes in the dark.
8. Unseal the plate, and add 100 µl Stop Solution to each well.
9. Measure the OD of each well with a microplate reader at 555 nm. Record these values as A_2. Use the $A_{2\ Standard}$ values for the standard curve.

C. Calculation of Results

Plot the standard curve, using the A_2 values of the standard dilutions (adjusted for the blank) on the y-axis, and their respective concentrations on the x-axis. The curve should form a straight line, described by the formula $y = ax + b$. Based on this curve, the activity of Glycerol Kinase in each sample well can be derived with the following formula:

1. Tissue samples:

One unit of Glycerol Kinase activity is defined as the amount required for 1 g of tissue protein to produce 1 µmol of glycerin-3-phosphate per minute at 37°C.

$$\text{Glycerol Kinase (U/g Protein)} = \frac{(\Delta A - b)}{a \times t \times C_{Protein}} \times F \times 1000$$

where:

ΔA	$A_{2\ Sample} - A_{1\ Sample}$
$A_{1\ Sample}$	OD value of sample before incubation
$A_{2\ Sample}$	OD value of sample after incubation
$A_{2\ Standard}$	OD value of standard after incubation
$C_{Protein}$	Concentration of protein in sample (g Protein/L)
a	Gradient of the standard curve ($y = \boldsymbol{a}x + b$)
b	Y-intercept of the standard curve ($y = ax + \boldsymbol{b}$)
t	Time of the enzymatic reaction (25 mins)
F	The dilution factor of sample
1000	1 mmol/L = 1000 µmol/L

Technical Support

For troubleshooting and technical assistance, please contact us at support@abbexa.com.

For Reference Only