Glyceraldehyde-3-Phosphate Dehydrogenase (GAPDH) Assay Kit

Catalog No.: abx294422

Size: 96 tests

Detection Range: 0.49 U/L – 50 U/L

Sensitivity: 0.49 U/L

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

Application: For detection and quantification of GAPDH activity in tissue homogenates and cell lysates.

Introduction

Gylyceraldehyde-3-Phosphate Dehydrogenase (GAPDH) is a key enzyme which catalyzes the sixth step of glycolysis. GAPDH catalyzes the reversible oxidation and phosphorylation of D-glyceraldehyde-3-phosphate into 1,3-bisphosphoglycerate, a crucial reaction in this metabolic pathway. Beyond its metabolic role, GAPDH is also involved in several other cellular functions, such as cytoskeletal organization and interactions with proteins linked to neurodegenerative disorders.

Abbexa's Glyceraldehyde-3-Phosphate Dehydrogenase Assay Kit is a quick, convenient, and sensitive method for measuring and calculating GAPDH activity. GAPDH catalyzes the oxidation of glyceraldehyde-3-phosphate, the product produced has an absorbance maximum at 450 nm. The change in OD value over time can therefore be used to calculate the GAPDH activity.

Kit components

1. 96-well microplate
2. Substrate Solution: 4 vials
3. Buffer Solution: 20 ml
4. Chromogenic Reagent: 3 ml
5. Standard: 2 vials
6. Plate sealer: 2

Materials required but not provided

1. Microplate reader (450 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. Centrifuge and centrifuge tubes
7. Mechanical homogenizer
8. Sonicator
9. Vortex mixer
10. Incubator

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 or -80°C for long-term storage. Avoid multiple freeze-thaw cycles.

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

- **Tissue Homogenates:** Carefully weigh at least 20 mg of tissue, and wash with cold PBS (0.01 M, pH 7.4). Per 20 mg of sample tissue, add 180 µl normal saline (0.9% NaCl). Homogenize manually, using a mechanical homogenizer at 4°C. Centrifuge at 10,000 × g for 10 minutes at 4°C, then carefully collect the supernatant and keep on ice for detection.
- **Cell lysates:** Collect at least 1 × 10^6 cells and wash with PBS (0.01 M, pH 7.4). Per 1 × 10^6 cells, add 200 µl normal saline (0.9% NaCl). Homogenize manually by ultrasonication at 4°C. Centrifuge at 10,000 × g for 10 minutes at 4°C, then carefully collect the supernatant and keep on ice for detection.

  Note: To calculate GAPDH activity in tissue homogenates or cell lysates using the formulae in section **C. Calculation of Results**, the total protein concentration of the supernatant must be determined separately (animal tissue: **abx097193**, plant tissue: **abx097194**, cell lysates: **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 lung tissue homogenate	1
10% Spinach tissue homogenate	1
10% Squash tissue homogenate	1
10% Cabbage tissue homogenate	1
1 × 10^6 HL-60 cells	1
1 × 10^6 CHO cells	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 cell lysates and tissue homogenates.

2. Reagents

- **Substrate Working Solution:** Add one vial of Substrate Solution into 2 ml of Buffer Solution and mix thoroughly. Transfer the solution to a 5 ml tube and dilute with 1 ml Buffer Solution. Mix thoroughly. Prepare immediately before use.
- **1 mmol/L Standard Solution:** Dissolve one vial of standard in 2 ml of double-distilled water and mix thoroughly to dissolve. Transfer the solution to a 10 ml tube and dilute with 3 ml double-distilled water. Mix thoroughly. The 1 mmol/L Standard Solution can be stored at -20°C for up to 2 weeks in the dark.
- **500 µmol/L Standard Solution:** Prepare enough 500 µmol/L Standard Solution for the number of wells to be used. Dilute the 1 mmol/L Standard Solution 1:1 with double distilled water. For example, mix thoroughly 500 µl 1 mmol/L Standard Solution and 500 µl double-distilled water to prepare 1000 µl of 500 µmol/L Standard Solution. The 500 µmol/L Standard Solution can be stored at -20°C for up to 3 days in the dark.
- **Standards:** Label 7 tubes with 500 µmol/L, 400 µmol/L, 350 µmol/L, 300 µmol/L, 250 µmol/L, 200 µmol/L, and 100 µmol/L. Prepare the standards according to the volumes as summarized in the following table:

Standard Dilution (µmol/L)	**500**	**400**	**350**	**300**	**250**	**200**	**100**
500 µmol/L Standard (µl)	200	160	140	120	100	80	40
Double-distilled water (µl)	0	40	60	80	100	120	160

For the blank 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.

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 the wells in duplicate.*
2. Add 40 µl of sample to each sample well.
3. Add 40 µl of each standard dilution to the corresponding standard wells.
4. Add 40 µl of double-distilled water to the blank wells.
5. Add 100 µl of Substrate Working Solution to all wells.
6. Add 20 µl of Chromogenic Reagent to all wells.
7. Mix thoroughly using a microplate shaker or by tapping the plate gently for 5 seconds.
8. Measure the OD value of each well at 450 nm using a microplate reader. Record these values as A_1.
9. Incubate at 37°C for 10 minutes in the dark, then measure the OD of each well at 450 nm using a microplate reader. Record these values as A_2.

Note: *Use the A_2 values of the standard wells 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 GAPDH in each sample well can be derived with the following formulae:

1. Tissue homogenates and Cell lysate samples:

One unit of GAPDH activity is defined as the amount of enzyme in 1 g of tissue or cell protein required to hydrolyze the substrate to produce 1 µmol of product per minute at 37°C.

$$\text{GAPDH Activity (U/g protein)} = \frac{(\mathrm{A_{2\ Sample}} - \mathrm{A_{1\ Sample}} - \mathrm{b})}{\mathrm{a} \times \mathrm{t} \times \mathrm{C_{Protein}}} \times \mathrm{F}$$

where:

$\mathrm{A_{2\ Sample}}$	OD value of sample after incubation
$\mathrm{A_{1\ Sample}}$	OD value of sample before incubation
$\mathrm{C_{Protein}}$	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 (10 minutes)
F	The dilution factor of sample

Technical Support

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