Glutamate Dehydrogenase Assay Kit

Catalog No.: abx298832

Size: 96 tests

Detection Range: 0.54 U/L – 25.0 U/L

Sensitivity: 0.54 U/L

Storage: Store all components in the dark at 2-8°C.

Application: For detection and quantification of glutamate dehydrogenase (GDH) activity in serum, plasma, tissue, and urine.

For Reference Only

Introduction

Glutamate dehydrogenase (GDH) is vital in carbon and nitrogen metabolism because it catalyzes the reversible oxidative deamination of glutamate to α-ketoglutaric acid. Glutamate dehydrogenase activity is an important indicator and diagnostic marker of hepatocyte cancer.

Abbexa's Glutamate Dehydrogenase Assay Kit is a quick, convenient, and sensitive method for measuring and calculating Glutamate Dehydrogenase activity. The product has an absorbance maxima at 450 nm. Glutamate dehydrogenase activity can be calculated by measuring the change of absorbance values at 450nm.

Kit components

1. 96-well microplate
2. Extraction Solution: 50 ml
3. Buffer solution: 15 ml
4. Substrate A: 1 vial
5. Substrate B: 5 ml
6. Detection Reagent: 2 × 1.2 ml
7. Standard: 2 vials
8. Plate sealer: 2

Materials Required But Not Provided

1. Microplate reader (450 nm)
2. Distilled water
3. Pipette and pipette tips
4. Vials/tubes
5. Timer
6. Sonicating water bath
7. Centrifuge
8. Vortex mixer
9. Incubator (37°C)

Protocol

A. Preparation of samples and reagents

1. Reagents

Bring all reagents to room temperature before use.

- **Substrate A Working Solution**: Dissolve a vial of Substrate A with 1.5 ml of double distilled water and keep on ice for detection. The prepared solution should be stored in the dark at 2-8°C for up to 7 days.
- **Substrate B Working Solution**: Mix Substrate A Working Solution and Substrate B at a 1:2 ratio. Prepare the needed amount just before use to ensure it is fresh. The prepared solution can be stored at room temperature for up to 12 hours.
- **Detection Reagent Working Solution**: Mix Buffer Solution and Detection Reagent at a 6:1 ratio. Prepare the needed amount just before use to ensure it is fresh. The prepared solution should be stored in the dark and can be used within 1 day.
- **1 mmol/L Standard Solution**: Dissolve a vial of Standard with 0.5 ml of double distilled water and preserve it on ice for detection. The prepared solution should be stored in the dark at 2-8°C for up to 7 days.

2. 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. Fresh samples are recommended.

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

- **Serum:** Samples should be collected into a serum separator tube. Coagulate the serum by leaving the tube undisturbed in a vertical position overnight at 4°C or at room temperature for up to 1 hr. Centrifuge at approximately 10000 × g for 10 mins at 4°C. If a precipitate appears, centrifuge again. Take the supernatant, keep on ice and assay immediately, or aliquot and store at -80°C for up to 1 month.
- **Plasma:** Collect plasma using heparin as the anticoagulant. Centrifuge for 10 mins at 10000 × g at 4°C, within 30 mins of collection. If precipitate appears, centrifuge again. Avoid hemolytic samples. Take the supernatant (avoid taking the middle layer containing white blood cells and platelets), keep on ice and assay immediately, or aliquot and store at -80°C for up to 1 month.
- **Tissue Homogenates:** Weigh 0.02-1 g of tissue. For each 1 g of tissue, add 9 ml of Extraction Solution. Homogenize by hand, using a mechanical homogenizer, or by ultrasonication on ice. Centrifuge the homogenate at 10000 x g at 4°C for 15 min. Collect the supernatant and assay immediately. The protein concentration in the supernatant should be determined separately.

Samples should not contain detergents such as SDS, Tween-20, NP-40 and Triton X-100, or reducing agents such as DTT.

It is recommended to carry out a preliminary experiment to determine the optimal dilution factor of samples before carrying out the formal experiment.

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The recommended dilution factor for different samples is as follows (for reference only):

Sample Type	Dilution Factor
10% Rat Liver Tissue Homogenate	3-5
10% Rat Kidney Tissue Homogenate	1
10% Rat Heart Tissue Homogenate	1
10% Rat Brain Tissue Homogenate	1
10% Rat Spleen Tissue Homogenate	1
10% Rat Lung Tissue Homogenate	1
10% Mouse Liver Tissue Homogenate	1
10% Mouse Kidney Tissue Homogenate	1
10% Mouse Spleen Tissue Homogenate	1
10% Mouse Lung Tissue Homogenate	1
Human Serum	1
Human Plasma	1
Rat Serum	1
Rat Plasma	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.
- The diluent is the Extraction Solution.

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B. Assay Procedure

1. Set control, standard and sample wells on the microplate and record their positions. It is recommended to use 2 control and 2 standards for each assay run. *Add the solution to the bottom of each well without touching the side walls. Pipette samples up and down to mix before adding to wells. Avoid foaming or bubbles.*
2. Dilute 1 mmol/L standard solution with Extraction Solution to create a serial concentration. The recommended dilution gradient is as follows: 0.00, 0.05, 0.10, 0.20, 0.25, 0.30, 0.40, and 0.50 mmol/L. Please see the below table as a reference.

Standard concentrations (mmol/L)	1 mmol/L standard solution (μL)	Extraction Solution (μL)
0.00	0	200
0.05	10	190
0.10	20	180
0.20	40	160
0.25	50	150
0.30	60	140
0.40	80	120
0.50	100	100

3. Add 20 μl of Standard Solution to the standard wells.
4. Add 20 μl of sample to the control wells.
5. Add 20 μl of sample to the sample wells.
6. Add 60 μl of double distilled water to the control wells.
7. Add 60 μl of Substrate B Working Solution to the standard wells
8. Add 60 μl of Substrate B Working Solution to the sample wells.
9. Add 140 μl Detection Reagent Working Solution to each well and mix fully.
10. Incubate in the dark at 37°C for 20 minutes.
11. Measure the OD of each well with a microplate reader at 450 nm.

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C. Calculation of Results

The standard curve can be plotted as the absolute OD_{450} of each standard solution (y) vs. the respective concentration of the standard solution (x). A linear fit is recommended for the standard curve (y = ax + b). Create the standard curve with graph software. The Glutamate Dehydrogenase concentration of the samples can be interpolated from the standard curve.

1. Serum and plasma:

One unit is defined as the amount of GDH in 1 L of liquid sample per 1 minute that hydrolyses the substrate to produce 1 µmol of product at 37°C.

$$\text{GDH activity (U/L)} = \frac{(\Delta A - b)}{a \times T} \times f \times 1000$$

2. Tissues samples:

One unit is defined as the amount of GDH in 1 g of tissue per 1 minute that hydrolyzes the substrate to produce 1 µmol at 37°C.

$$\text{GDH activity (U/gprot)} = \frac{(\Delta A - b)}{a \times T \times C_{pr}} \times f \times 1000$$

where:

y	$OD_{Standard} - OD_{Blank}$
x	The concentration of standard
a	The slope of standard curve
b	The intercept of standard curve
ΔA	OD_{Sample} - $OD_{Control}$
T	The time of incubation reaction, 20 minutes
C_{pr}	Concentration of the protein in sample (gprot/L)
f	Dilution factor of sample before assay
C_{Sample}	The concentration of sample (g/L)
1000	Unit conversion: 1 mmol/L = 1000 µmol/L

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