Glutamate dehydrogenase (GDH) Activity Assay Kit

Note: Take two or three different samples for prediction before test.

Operation Equipment: Spectrophotometer/ Microplate reader

Cat No: BC1465

Size: 100T/96S

Components:

Extract solution: 100mL×1. Storage at 4℃.

Reagent 1: 20mL×1. Storage at 4℃.

Reagent 2: Powder×1. Storage at -20℃.

Product Description:

GDH (EC 1.4.1.2) is widely distributed in plants. GDH and glutamate synthetase (GOGAT) are involved in the synthesis of glutamate. It plays an important role in the assimilation of ammonia and the metabolism of organic nitrogen compounds.

GDH catalyzes the NH_4^+, α-ketoglutaric acid and NADH to form glutamic acid and NAD^+, which cause a decrease in absorbance at 340 nm. The GDH activity is calculated by measuring the rate of decrease in absorbance at 340 nm.

Reagents and Equipment Required but Not Provided:

Spectrophotometer/Microplate reader, desk centrifuge, adjustable pipette, water bath, micro quartz cuvette/ 96-well flat-bottom plate (UV), mortar/homogenizer, ice and distilled water.

Sample preparation:

1.Cells or bacterial: Collecting bacteria or cells into a centrifuge tube, discard supernatant after centrifugation. Suggest 5 million with 1mL of Extract Solution. Use ultrasonic to split bacteria or cells (power 20%, work time 3s，interval 10s，repeat for 30 times). Centrifuge at 8000 g for 10 min at 4℃. Supernatant is placed on ice for test.

2.Tissue: Add 1 mL of Extract solution into 0.1g of tissue, fully grinding on ice. Centrifuge at 8000 g for 10 min at 4℃. supernatant on ice is used for test.

Procedure:

1. Preheat spectrophotometer/microplate reader for 30 min, adjust the wavelength to 340 nm, set the counter to zero with distilled water.
2. Sample determination

(1) Preparation of working solution: before use, the Reagent 2 is added to the 19 mL of Reagent 1 to be dissolved, and placed in a water bath at 37°C (mammal) or 25°C (other species) for 5 min;

(2) Take 0.19 mL of working solution and 0.01 mL of sample in a micro quartz cuvette /96-well flat-bottom plate (UV), mix well, start timing while adding the sample, and record the initial absorbance A1 of 20s and A2 of 320s at 340 nm. ΔA = A1 - A2.

Calculation:

1. Micro cuvette:

(1) Protein concentration:

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the consumption of 1nmol of NADH per min every milligram of protein.

GDH (U/mg prot) = ΔA÷(ε×d) ×Vrv×10^9÷(Vs×Cpr)÷T =643×ΔA÷Cpr

(2) Sample weight:

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the consumption of 1nmol of NADH per min every gram of tissue.

GDH (U/g) = ΔA÷(ε×d) ×Vrv×10^9÷(Vs÷Vsv×W) ÷T=643×ΔA÷W

(3) Cells or bacterial:

Unit definition:One unit of enzyme activity is defined as the amount of enzyme catalyzes the consumption of 1nmol of NADH per min 10^4 cells.

GDH (U/mL) = ΔA÷(ε×d) ×Vrv×10^9÷(Vs÷Vsv×500)÷T=1.286×ΔA

ε: tyrosine molar extinction coefficient, 6220 L/mol/cm;

d: light path of cuvette, 1cm;

Vrv: total reaction volume,0.0002L;

Vs: supernate volume (mL), 0.01mL;

Cpr: crude enzyme protein concentration (mg/mL); need to detect separately.

T: Reaction time (min), 5min;

W: Sample weight (g);

Vsv: Extraction volume, 1 mL;

500: Number of cells, 5 million cells

2. 96-well flat-bottom plate:

(1) Protein concentration:

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the consumption of 1nmol of NADH per min every milligram of protein.

GDH (U/mg prot)= ΔA÷(ε×d)×Vrv×10^9÷(Vs×Cpr)÷T =1072×ΔA÷Cpr

(2) Sample weight:

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the consumption of 1nmol of NADH per min every gram of tissue.

GDH (U/g) = ΔA÷(ε×d) ×Vrv×10^9÷(Vs÷Vsv×W)÷T=1072×ΔA÷W

(3) Cells or bacterial:

Unit definition:One unit of enzyme activity is defined as the amount of enzyme catalyzes the consumption of 1nmol of NADH per min 10^4 cells.

GDH (U/mL)= ΔA÷(ε×d)×Vrv×10^9÷(Vs÷Vsv×500)÷T=2.144×ΔA

ε: tyrosine molar extinction coefficient, 6220 L/mol/cm;

d: light path of cuvette, 0.6 cm;

Vrv: total reaction volume,0.0002L;
Vs: supernate volume (mL), 0.01mL;
Cpr: crude enzyme protein concentration (mg/mL); need to detect separately.
T: Reaction time (min), 5min;
W: Sample weight (g);
Vsv: Extraction volume, 1 mL;
500: Number of cells, 5 million cells

Note

1. When ΔA is greater than 0.5, the sample shall be diluted for measurement.
2. Because the Extract solution contains a certain concentration of protein (about 1mg/mL), the protein content of the Extract solution itself needs to be subtracted when determining the protein concentration of the sample.

Recent Product citations：

[1] Fei Ding,Qiannan Hu,Meiling Wang,et al. Knockout of SlSBPASE Suppresses Carbon Assimilation and Alters Nitrogen Metabolism in Tomato Plants. International Journal of Molecular Sciences. December 2018;(IF4.183)

[2] Lin Y, Nan J, Shen J, et al. Canagliflozin impairs blood reperfusion of ischaemic lower limb partially by inhibiting the retention and paracrine function of bone marrow derived mesenchymal stem cells[J]. EBioMedicine, 2020, 52: 102637.

References：

[1] Wen J F, Gong M, Liu Y, et al. Effect of hydrogen peroxide on growth and activity of some enzymes involved in proline metabolism of sweet corn seedlings under copper stress[J]. Scientia horticulturae, 2013, 164: 366-371.

Related Products:

BC0080/BC0085 Nitrate reductase(NR)Activity Assay kit
BC1450/BC1455 Glutaminase (GLS) Assay Kit