EZMeta™ Glutamic Acid Dehydrogenase (GDH) Colorimetric Activity Kit

Cat #: D-AKC3041

Size: 48T / 96T

Storage: Stored at 4°C for 12 months, protected from light

Product Information

Applicable samples: Animal and Plant Tissue, Cell, Bacteria, Serum, Plasma

Assay Principle

Glutamate (Glu) is widely found in animals, plants, microorganisms and cultured cells, and is one of the main sources of amino groups in organisms. Glutamate dehydrogenase (GDH) can catalyze the reversible oxidative dehydrogenation of glutamate to α-ketoglutarate, which is a key enzyme in carbon and nitrogen metabolism. GDH together with glutamate synthase (GOGAT), participates in the synthesis of glutamate and plays an important role in ammonia assimilation and conversion to organic nitrogen compounds. EZMeta™ Glutamic Acid Dehydrogenase (GDH) Colorimetric Activity Kit provides a simple, convenient and rapid GDH activity detection method, which is suitable for the detection of animal and plant tissue, cell, bacteria, serum, plasma and other samples. The detection principle is that GDH catalyzes $NH4^+$, NADH and α-ketoglutaric acid to produce glutamic acid and NAD^+, which leads to the decrease of absorbance of 340 nm. By measuring the decreasing rate of absorbance of 340 nm, the activity of GDH is calculated.

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	48 T	96 T	
Reagent Ⅰ	50 mL	100 mL	4°C
Reagent Ⅱ	10 mL	20 mL	4°C
Reagent Ⅲ	1	2	4°C, protected from light

Materials Required but Not Supplied

·Microplate reader or ultraviolet spectrophotometer capable of measuring absorbance at 340 nm

·96-well UV plate or microquartz cuvette, precision pipettes, disposable pipette tips

·Ice maker, refrigerated centrifuge

·Deionized water

·Homogenizer (for tissue samples)

Reagent Preparation

Reagent Ⅰ: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4°C.

Working Solution: Before use, Add 9 mL Reagent Ⅱ to ReagentⅢ, mix it well and prepare for use. Store at 4°C, protected from light.

Sample Preparation

Note: Fresh samples are recommended. All samples and reagents should be on ice to avoid denaturation and deactivation.

1. Tissues: Weigh 0.1 g tissue, add 1 mL Reagent Ⅰ and homogenize on ice. Centrifuge at 8,000 g for 10 min at 4°C. Use supernatant for assay, and place it on ice to be tested.

2. Cell (bacteria): Collect 5×10^6 cell or bacteria into the centrifuge tube, wash with cold PBS, discard the supernatant after centrifugation; add 1 mL Reagent Ⅰ to ultrasonically disrupt the bacteria 5 min (power 20% or 200 W, ultrasonic 3 s, interval 7 s, repeat 30 times). Centrifuge at 8,000 g for 10 min at 4°C. Use supernatant for assay, and place it on ice to be tested.

3. Serum (Plasma): Direct detection.

Note: It will be better to quantify the total protein with EZMeta™ Glutamic Acid Dehydrogenase (GDH) Colorimetric Activity Kit, if the content is calculated by protein concentration.

Assay Procedure

1. Preheat the microplate reader or ultraviolet spectrophotometer for more than 30 min, and adjust the wavelength to 340 nm, ultraviolet spectrophotometer was returned to zero with deionized water.

2. Working Solution was incubated at 37°C (mammals) or 25°C (other species) for 30 min.

3. Sample measurement. (The following operations are operated in the 96-well UV plate or microquartz cuvette)

Reagent	Test Well (μL)
Sample	10
Working Solution	190

4. After mixing quickly, record the absorbance values of 20 s and 5 min 20 s at 340 nm, mark as A_1 and A_2, and calculate $\Delta A_{Test}=A_{Test1}-A_{Test2}$.

Note: In order to guarantee the accuracy of experimental results, need to do a pre-experiment with 2-3 samples. If ΔA_{Test} is greater than 0.5, the sample can be appropriately diluted with Reagent Ⅰ, the calculated result multiplied by the dilution factor. It is not suggested to test too many samples at the same time, because enzyme activity is calculated by the variation of absorbance value per unit time.

Data Analysis

Note: We provide you with calculation formulae, including the derivation process and final formula. The two are exactly equal. It is suggested that the concise calculation formula in bold is final formula.

A. 96-well UV plates calculation formula

1. Calculation of GDH activity in serum

Unit definition: One enzyme activity unit defines as 1 nmol NADH consumed by each mL of serum per min.

GDH (U/mL)=[$\Delta A_{Test} \times V_{Total} \div (\varepsilon \times d) \times 10^9$]$\div V_{Sample} \div T$**=1,286×$\Delta A_{Test}$**

2. Calculation of GDH activity in tissue of the sample

(1) Calculation according to the protein concentration of the sample

Unit definition: One enzyme activity unit defines as 1 nmol NADH consumed by 1 mg tissue proteins per min.

GDH (U/mg prot)=[$\Delta A_{Test} \times V_{Total} \div (\varepsilon \times d) \times 10^9$]$\div (V_{Sample} \times Cpr) \div T$**=1,286×$\Delta A_{Test}$÷Cpr**

(2) Calculation according to the weight of the sample

Unit definition: One enzyme activity unit defines as 1 nmol NADH consumed by 1 g tissue per min.

GDH (U/g weight)=[$\Delta A_{Test} \times V_{Total} \div (\varepsilon \times d) \times 10^9$]$\div (W \div V_{Reagent\ I} \times V_{Sample}) \div T$**=1,286×$\Delta A_{Test}$÷W**

3. Calculation of GDH activity in cell or bacteria

Unit definition: One enzyme activity unit defines as 1 nmol NADH consumed by 10^4 cells or bacteria per min.

GDH (U/10^4)=[$\Delta A_{Test} \times V_{Total} \div (\varepsilon \times d) \times 10^9$]$\div (V_{Sample} \div V_{Reagent\ I} \times 500) \div T$**=2.572×$\Delta A_{Test}$**

Where: V_{Total}: the total volume of the reaction system, 0.2 mL=2×10^{-4} L; $V_{Reagent\ I}$: the volume of the Reagent Ⅰ, 1 mL; V_{Sample}: the volume of the supernatant in the reaction system, 0.01 mL; ε: NADH molar extinction coefficient, 6.22×10^3 L/mol/cm; d: 96-well UV plate diameter, 0.5 cm; Cpr: protein concentration (mg/mL); T: reaction time, 5 min; W: sample weight, g; 500: total number of cells or bacteria, 5 million; 10^9: unit conversion factor, 1 mol=10^9 nmol.

B. Microquartz cuvette calculation formula

The optical diameter d:0.5 cm in the above calculation formula can be adjusted to d:1 cm for calculation.

Disclaimer

The reagent is only used in the field of scientific research, not suitable for clinical diagnosis or other purposes.