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Glyceraldehyde-3-phosphate Dehydrogenase(GAPDH) Activity Assay Kit

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

Detection instrument: Spectrophotometer/ Microplate reader

Cat No: AK0241

Size: 100T/96S

Components:

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

Reagent I: Powder×1. Store at -20℃ .

Reagent II: 20 mL×1. Store at 4℃ .

Reagent III: 12 μL×1. Store at 4℃ . The liquid is placed in the EP tube in the reagent bottle. According to the dosage and the volume ratio of Reagent III: distilled water of 3:100, mix well, use and prepare now.

Product Description:

GAPDH (EC 1.2. 1. 12) catalyzes the oxidation of glyceraldehyde 3-phosphate to 1,3-diphosphoglyceride. It is the key enzyme of glycolysis pathway. It is closely related to the pathway of gluconeogenesis, the maintenance of blood glucose concentration and the occurrence of diabetes. It plays an important role in the disorders of glucose, lipid and protein metabolism.

3-phosphoglycerate kinase can catalyze the production of 1,3-diphosphoglyceride from triphosphate and ATP. GAPDH reversely catalyzes the formation of glyceraldehyde-3-phosphate, inorganic phosphorus and NAD^+ from 1,3-diphosphoglyceride and NADH. The decrease of NADH measured at 340 nm can reflect the activity of GAPDH.

Required material

Desk centrifuge, ultraviolet spectrophotometer/microplate reader, constant temperature water bath, mortar/ homogenizer, micro quartz cuvette/96 well UV plate, transferpettor, ice and distilled water.

Procedure:

I. Sample Extraction:

1. Tissue sample:

According to the mass of the tissue (g): the volume of the Extract solution (mL) is 1: 5~10. Suggested 0.1 g of tissue with 1 mL of Extract solution. Fully grind on ice, centrifuge at 8000 g and 4℃ for 20 min. Supernatant is placed on ice for test.

2. Bacteria or cells:

According to the number of cells (10^4): the volume of the Extract solution (mL) is 500 ~ 1000: 1. Suggest 5 million with 1 mL of Extract Solution. Use ultrasonication to split bacteria or cells (power 20% or 200W, work time 3s， interval 10s， repeat for 30 times). Centrifuge at 8000 g and 4℃ for 10 min. Supernatant is placed on ice for test.

3. Serum (plasma): direct measurement.

II. Determination procedure:

1 Preheat the ultraviolet spectrophotometer/microplate reader 30 min, adjust wavelength to340 nm, set zero with distilled water.

2 Preparation of working solution: pour all Reagent II into one bottle of Reagent I. Fully dissolved. Preheat a certain amount of 37℃ (mammal) or 25℃ (other species) for 10 min as required. The unused reagents shall be stored at -20℃ after sub charging. Avoid repeated freezing and thawing.

3 Add reagents with the following list:

Reagent name (μL)	Test tube (T)	Blank tube (B)
Sample	6	
Distilled water		6
Reagent III	4	4
Working solution	190	190

Add the above reagents into the micro quartz cuvette/96 well UV plate respectively. Mix thoroughly. Measure the absorbance value A1 at 340 nm for 10s. Quickly put it into a water bath or incubator at 37℃ (mammal) or 25℃ (other species) for 5 min (the temperature can be adjusted to 37℃ or 25℃ with the temperature control function of the microplate reader). Take out and dry it quickly. Measure the absorbance value A2 for 5min10s. Calculate $\Delta A_T = A1_T - A2_T$, $\Delta A_B = A1_B - A2_B$, $\Delta A = \Delta A_T - \Delta A_B$. Blank tube only needs to test 1-2 times.

III. Calculation:

1 Calculated by micro quartz cuvette

1) Calculated by protein concentration:

Unit definition: One unit of enzyme activity is defined as the amount of enzymes consume of 1 nmol of NADH in the reaction system per minute every mg protein.

GAPDH activity (U/mg prot) $=\Delta A \div (\varepsilon \times d) \times V_{RV} \times 10^9 \div (V_S \times Cpr) \div T = 1072 \times \Delta A \div Cpr$

2) Calculated by sample weight

Unit definition: One unit of enzyme activity is defined as the amount of enzymes consume of 1 nmol of NADH in the reaction system per minute every g sample.

GAPDH activity (U/g fresh weight) $=\Delta A \div (\varepsilon \times d) \times V_{RV} \times 10^9 \div (V_S \times W \div V_{ST}) \div T = 1072 \times \Delta A \div W$

3) Calculated by bacteria or cell amount:

Unit definition: One unit of enzyme activity is defined as the amount of enzymes consume of 1 nmol of NADH in the reaction system per minute every 10^4 bacteria or cells.

GAPDH activity ($U/10^4$ cell) $=\Delta A \div (\varepsilon \times d) \times V_{RV} \times 10^9 \div (V_S \times 500 \div V_{ST}) \div T = 2.14 \times \Delta A$

4) Calculated by volume of culture medium:

Unit definition: One unit of enzyme activity is defined as the amount of enzymes consume of 1 nmol of NADH in the reaction system per minute every mL liquid.

GAPDH activity (U/mL) $=\Delta A \div (\varepsilon \times d) \times V_{RV} \times 10^9 \div V_S \div T = 1072 \times \Delta A$

ε: molar extinction coefficient of NADH: 6.22×10^3 L/mol/cm;

d: light path of cuvette, 1 cm;

V_{RV}: total reaction volume, 0.0002 L;
V_S: sample volume in reaction system, 0.006 mL;
V_{ST}: volume of extraction solution added, 1 mL;
Cpr: sample protein concentration, mg/mL;
W, sample mass, g;
T: reaction time, 5 min;
10^9: conversion factor, 1 mol = 10^9 nmol;
500: Number of cells, 5 million.

2. Calculated by 96 well UV plate:

Modify d- 1 cm in the above formula to d-0.6 cm (light path of the cuvette) for calculation.

Note:

1. When A1 is less than 0.8 or ΔA is greater than 0.7 (for 96 well UV plate, A1 is less than 0.4 or ΔA is greater than 0.4), it is recommended to dilute the sample before determination.

2. The blank tube is a test tube for testing the quality of each reagent component. Under normal conditions, the change does not exceed 0.02.

Experimental example:

1. Take 0. 1g of rabbit kidney, add 1 mL of Extract solution, homogenize in ice bath, then centrifuge at 8000g, 4℃ for 20 min, take the supernatant and put it on ice, then operate with micro quartz cuvette according to the determination steps, measure and calculate: $\Delta A_T = A1_T - A2_T = 0.9333 - 0.2289 = 0.7044$, $\Delta A_B = A1_B - A2_B = 0.9322 - 0.9274 = 0.0048$, $\Delta A = \Delta A_T - \Delta A_B = 0.6996$.

GAPDH activity (U / g mass) = 1072×ΔA ÷ W = 7499.7 U/g mass.

2. Take 0. 1g of clover, add 1 mL of Extract solution, homogenize it in ice bath, then centrifuge it in 8000g and 4℃ for 20 min, take the supernatant and put it on ice, then operate according to the determination steps, measure and calculate it with micro quartz cuvette, $\Delta A_T = A1_T - A2_T = 1.0184 - 1.0042 = 0.0142$, $\Delta A_B = A1_B - A2_B = 0.9322 - 0.9274 = 0.0048$, $\Delta A = \Delta A_T - \Delta A_B = 0.0094$.

GAPDH activity (U/g mass) = 1072 × ΔA ÷ W = 100.77 U/g mass.

3. Take rabbit serum for direct detection, measure with micro quartz colorimetric plate and calculate $\Delta A_T = A1_T - A2_T = 0.9528 - 0.9303 = 0.0225$, $\Delta A_B = A1_B - A2_B = 0.9322 - 0.9274 = 0.0048$.

GAPDH activity (U/mL) = 1072 × ΔA = 18.974 U/mL.

Related Products：

AK0302/AK0299 Plant Chlorophyll Content Assay Kit
AK0080/AK0079 Plant Carotenoid Content Assay Kit