Glycogen Synthetase (GCS) Assay Kit

Note: It is necessary to predict 2-3 large difference samples before the formal determination.

Operation Equipment: Spectrophotometer/microplate reader

Cat No: NA0391

Size: 100T/96S

Components:

Extract solution: Liquid 100 mL×1, store at 4°C;

Reagent I: Liquid 18 mL×1, store at 4°C;

Reagent II: Liquid 7.5 mL×1, store at 4°C;

Reagent III: Liquid 14 μL×1, store at 4°C and protect from light;

Reagent IV: Powder×1, store at -20°C;

Reagent V: Powder×1, store at -20°C;

Reagent VI: Liquid 48 μL×1, store at 4°C and protect from light;

Reagent VII: Powder×1, store at -20°C;

Reagent VIII: Powder×1, store at 4°C and protect from light;

Working solution: Transfer reagent III, IV and V to reagent I for mixing and dissolving before use; The rest of reagent can store at -20°C for one week; Avoid repeated freezing and thawing;

Preparation of reagent VIII: Add 5 mL of reagent II into reagent VIII and dissolve it before use. Then transfer reagent VI and VII to reagent VIII to mix and dissolve them for use; The rest of reagent can store at -20°C for one week; Avoid repeated freezing and thawing;

Product Description:

Glycogen synthetase (GCS) can add the glycogen of UDPG to the original glycogen or the non-reducing end of glycogen protein, and be connected by α-1,4 glycoside bond. GCS is the rate limiting enzyme of glycogen synthesis in animal body. It is also the main target enzyme of insulin. It plays an important role in the process of glucose metabolism and maintaining the relative stability of blood glucose.

GCS catalyzes the production of glycogen and UDP from UDPG and glucose residues. Pyruvate kinase and lactate dehydrogenase further catalyze NADH to generate NAD^+. The decrease rate of NADH at 340 nm can reflect the activity of GCS.

Required but Not Provided:

Ultraviolet spectrophotometer/microplate reader, balance, low temperature desk centrifuge, water-bath, transferpettor, micro quartz cuvette/96 well flat-bottom UV plate, EP tube, mortar/homogenizer, ice and distilled water.

Protocol

I. Preparation:

1. Tissue: according to the ratio of mass (g): extraction volume (mL): 1:5-10 to add the extract. It is suggested that add 1 mL of extract to 0.1 g of tissue. Homogenate on ice. Centrifuge at 8000 g 4℃ for 10 min. Take the supernatant on ice for test.
2. Bacteria and cells: according to the ratio of 10^4 cells: extract volume (mL) 500-1000:1. It is suggested to take about 500 million bacteria/cell and add 1 mL extraction reagent. Bacteria/cell is split by ultrasonication (power 200w, ultrasonic 3s, interval 7s, total time 3 min). Centrifuge at 8000 g 4℃ for 10 min. Take the supernatant on ice for test.
3. Serum and other liquids: detect directly.

II. Determination procedure:

1. Preheat ultraviolet spectrophotometer for 30 min, adjust wavelength to 340 nm, set the counter to zero with distilled water.
2. Operation table: (add the following reagents in micro quartz cuvette/96 well UV plate in turn)

Reagent (μL)	Test tube (A_T)	Blank tube (A_B)
Sample	10	
Distilled water		10
Reagent VIII	40	40
Working solution	150	150
Add samples and start timing. Mix thoroughly. The absorbance value A1 for 10s and A2 for 1 min10s be measured at 340 nm. Calculate ΔA_T=$A1_T$-$A2_T$, ΔA_B=$A1_B$-$A2_B$, ΔA= ΔA_T-ΔA_B. Blank tube only needs to be test once or twice.		

III. GCS Calculation:

a. Micro quartz cuvette

1) Protein concentration:

Unit definition: One unit of enzyme is defined as the amount of enzyme that catalyzes the consumption of 1 nmol NADH per minute every mg tissue protein in the reaction system.

GCS (U/mg prot) $=\Delta A\div(\varepsilon\times d)\times V_T\times10^9\div(Cpr\times V_{SA})\div T= 3215.4\times\Delta A\div Cpr$

2) Sample weight:

Unit definition: One unit of enzyme is defined as the amount of enzyme that catalyzes the consumption of 1 nmol NADH per minute every gram tissue weight in the reaction system.

GCS (U/g weight) $=\Delta A\div(\varepsilon\times d)\times V_T\times10^9\div(W\times V_{SA}\div V_E)\div T= 3215.4\times\Delta A\div W$

3) Cells

Unit definition: One unit of enzyme is defined as the amount of enzyme that catalyzes the consumption of 1 nmol NADH per minute every 10^4 cells in the reaction system.

GCS (U/10^4 cell) $=\Delta A\div(\varepsilon\times d)\times V_T\times10^9\div(\text{cells }(10^4)\times V_{SA}\div V_E)\div T= 3215.4\times\Delta A\div\text{cells }(10^4)$

4) Liquid volume

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

GCS(U/mL) $=\Delta A\div(\varepsilon\times d)\times V_T\times10^9\div V_{SA}\div T= 3215.4\times\Delta A$

ε: NADH molar extinction coefficient, 6.22×10^3 L/mol/cm;
d: Light path of cuvette, 1 cm;
10^9: Unit conversion coefficient, 1 mol = 10^9 nmol;
V_T: Total volume of reaction system, 2×10^{-4} L;
V_{SA}: Sample volume, 0.01 mL;
Cpr: Protein concentration, mg/mL;
W: Sample weight, g;
V_E: Extract solution volume of cells, 1 mL;
T: Reaction time, 1 min;

b. 96 well flat-bottom plate

The optical diameter d=1 cm of the cuvette in the above formula is changed to 0.6 cm of the 96 well UV plate.

Note:

1. The supernatant of sample extraction should be placed on ice for testing. It is recommended to finish the test on the same day.
2. If the ΔA is greater than 0.2, it is recommended to dilute the sample with the extract for determination. To improve the detection sensitivity. Multiply the corresponding dilution ratio in the calculation formula.

Experimental examples:

1. Take 0.1 g of mouse heart tissue and add 1 mL of Extract solution for sample processing. After centrifugation to take the supernatant, proceed according to the determination procedure. After determination with micro quartz cuvette, calculate $\Delta A_T = A1_T - A2_T$ =1.2455-1.1883=0.0572, $\Delta A_B = A1_B - A2_B$=0.9639-0.9529=0.011, $\Delta A = \Delta A_T - \Delta A_B$ =0.0572-0.011=0.0462. The enzyme activity is calculated according to the sample mass.
   GCS (U/g weight) = 3215.4×ΔA÷W=1485.5 U/g weight.

Related products:

NA0838/NA0596 β-1,3-glucanase(β-1,3-GA) Activity Assay Kit
NA0682/NA0441 Acidic Xylanase Activity Assay Kit
NA0312/NA0311 N-Acetyl-β-D-Glucosidase(NAG) Activity Assay Kit