

Glycogen Synthetase (GCS) Assay Kit

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

Operation Equipment: Ultraviolet Spectrophotometer

Cat No: BC3330

Size: 50T/48S

Components:

Extract solution: Liquid 60 mL×1, store at 4°C;

Reagent I: Liquid 45 mL×1, store at 4°C;

Reagent II: Liquid 15 mL×1, store at 4°C;

Reagent III: Liquid 40 μL×1, store at 4°C;

Reagent IV: Powder×2, store at -20°C; Provide a 5 mL reagent bottle, take 1 bottle and add 3 mL of reagent I before use to fully dissolve. Pack and store at -20°C for 4 weeks, avoid repeated freezing and thawing;

Reagent V: Powder×2, store at -20°C; Provide a 5 mL reagent bottle, take 1 bottle and add 3 mL of reagent I before use to fully dissolve. Pack and store at -20°C for 4 weeks, avoid repeated freezing and thawing;

Reagent VI: Liquid 115 μL×1, store at 4°C;

Reagent VII: Powder×2, store at -20°C;

Reagent VIII: Powder×2, store at 4°C and protect from light;

Working solution: Centrifuge Reagent III before use, take 20 μL of Reagent III, add 14 mL of Reagent I, 3 mL of Reagent IV, and 3 mL of Reagent V, mix thoroughly (about 26T), use it now, or prepare it in proportion to the sample volume;;

Preparation of reagent VIII:

(1) Before use, take 1 bottle of Reagent VIII and add 6mL of Reagent II to fully dissolve, then transfer 1 bottle of Reagent VII to Reagent VIII and mix and dissolve before use. It can be stored at -20°C for 4 weeks, avoiding repeated freezing and thawing. ;

(2) Centrifuge Reagent VI before use, add 55 μL of Reagent VI to 5.74 mL of the solution in above (1), mix thoroughly (about 28T), and prepare it for immediate use, or it can be prepared in proportion to the sample size.

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, low temperature benchtop centrifuge, water bath, 1mL quartz cuvette, adjustable pipette, sonicator, 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 solution. It is suggested that add 1 mL of extract to 0.1 g of tissue. Homogenate on ice. Centrifuge at 8000 g 4°C for 10 min. Take the supernatant on ice for test.
2. Bacteria and cells: according to the ratio of 10⁴ 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°C for 10 min. Take the supernatant on ice for test.
3. Serum and other liquids: detect directly. (If the solution is turbid, take the supernatant after centrifugation for measurement)

II. Determination procedure:

1. Preheat ultraviolet spectrophotometer for 30 min, adjust wavelength to 340 nm, set the counter to zero with distilled water.
2. Preheat the working solution and reagent VIII in a 37°C water bath for 5 minutes before use (how much the working solution is used to preheat).
3. Operation table: (add the following reagents in 1 mL quartz cuvette in turn)

Reagent (μL)	Test tube (A _T)	Blank tube (A _B)
Sample	50	
Distilled water		50
Working solution	750	750
Reagent VIII	200	200

Add samples and start timing. Mix thoroughly. The absorbance value A₁ for 10s and A₂ for 1 min10s be measured at 340 nm. Calculate $\Delta A_T = A_{1T} - A_{2T}$, $\Delta A_B = A_{1B} - A_{2B}$, $\Delta A = \Delta A_T - \Delta A_B$. Blank tube only needs to be test once or twice.

III. GCS Calculation:

- 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 milligram tissue protein in the reaction system.

$$\text{GCS (U/mg prot)} = \Delta A \div (\epsilon \times d) \times V_T \times 10^9 \div (C_{pr} \times V_{SA}) \div T = 3215.4 \times \Delta A \div C_{pr}$$

- 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.

$$\text{GCS (U/g weight)} = \Delta A \div (\epsilon \times d) \times V_T \times 10^9 \div (W \times V_{SA} \div V_E) \div T = 3215.4 \times \Delta A \div W$$

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.

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

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.

$$\text{GCS (U/mL)} = \Delta A \div (\epsilon \times d) \times V_T \times 10^9 \div V_{SA} \div T = 3215.4 \times \Delta A$$

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, 1×10^{-3} L;

V_{SA} : Sample volume, 0.05 mL;

Cpr: Protein concentration, mg/mL;

W: Sample weight, g;

V_E : Extract solution volume of cells, 1 mL;

T: Reaction time, 1 min;

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. Calculate $\Delta A_T = A_{1T} - A_{2T} = 1.177 - 1.119 = 0.058$, $\Delta A_B = A_{1B} - A_{2B} = 0.944 - 0.939 = 0.005$, $\Delta A = \Delta A_T - \Delta A_B = 0.058 - 0.005 = 0.053$. The enzyme activity is calculated according to the sample mass.

$$\text{GCS (U/g weight)} = 3215.4 \times \Delta A \div W = 1704.162 \text{ U/g weight.}$$

Related products:

BC0360/BC0365 β -1,3-glucanase(β -1,3-GA) Activity Assay Kit

BC2600/BC2605 Acidic Xylanase Activity Assay Kit

BC4290/BC4295 N-Acetyl- β -D-Glucosidase(NAG) Activity Assay Kit