Glycogen Synthetase (GCS) Assay Kit

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

Operation Equipment: Ultraviolet Spectrophotometer/microplate reader

Cat No: BC3335

Size: 100T/96S

Components:

Extract solution: Liquid 110 mL×1, store at 4℃;

Reagent I: Liquid 25 mL×1, store at 4℃;

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

Reagent III: Liquid 20 μL×1, store at 4℃;

Reagent IV: Powder×2, store at -20℃; Take 1 bottle and add 1.5 mL of reagent I before use to fully dissolve. Pack and store at -20℃ for 4 weeks, avoid repeated freezing and thawing;

Reagent V: Powder×2, store at -20℃; Take 1 bottle and add 1.5 mL of reagent I before use to fully dissolve. Pack and store at -20℃ for 4 weeks, avoid repeated freezing and thawing;

Reagent VI: Liquid 45 μL×1, store at 4℃;

Reagent VII: Powder×2, store at -20℃;

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

Working solution: Centrifuge Reagent III before use, take 10 μL of Reagent III, add 7 mL of Reagent I, 1.5 mL of Reagent IV, and 1.5 mL of Reagent V, mix thoroughly (about 66T), 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 2.5 mL 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℃ for 4 weeks, avoiding repeated freezing and thawing;

(2) Centrifuge Reagent VI before use, add 14 μL of Reagent VI to 1.46 mL of the solution in above (1), mix thoroughly (about 36T), 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/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. (If the solution is turbid, take the supernatant after centrifugation for measurement)

II. Determination procedure:

1. Preheat ultraviolet spectrophotometer/microplate reader for 30 min, adjust wavelength to 340 nm, ultraviolet spectrophotometer 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 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 $\Delta A_T=A1_T-A2_T$, $\Delta A_B=A1_B-A2_B$, $\Delta A=\Delta A_T-\Delta A_B$. Blank tube only needs to 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) $=\Delta A\div(\varepsilon\times d)\times V_T\times 10^9\div(Cpr\times V_{SA})\div T= 3215.4\times\Delta A\div Cpr$

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) =ΔA÷(ε×d)×VT×10^9÷(W×V_{SA}÷V_E)÷T= 3215.4×ΔA÷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 104 cells in the reaction system.

GCS (U/10^4 cell) =ΔA÷(ε×d)×VT×10^9÷(cells (10^4)×V_{SA}÷V_E)÷T= 3215.4×ΔA÷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) =ΔA÷(ε×d)×V_T×10^9÷V_{SA}÷T= 3215.4×ΔA

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 ΔA_T = $A1_T$-$A2_T$ =1.2455-1.1883=0.0572, ΔA_B = $A1_B$-$A2_B$=0.9639-0.9529=0.011, ΔA = ΔA_T-ΔA_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:

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

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