Glycerol-3-Phosphate Oxidase Activity Assay Kit

Note: Before the experiment, it is recommended to select 2-3 sample with large expected differences for pre-experiment.

Operation equipment: Spectrophotometer/Microplate reader

Cat No: BC6125

Size: 100T/96S

Product Composition: Before use, please carefully check whether the volume of the reagent is consistent with the volume in the bottle. If you have any questions, please contact Solarbio staff in time.

Reagent name	Size	Preservation Condition
Extract solution	Liquid 110 mL×1	2-8℃
Reagent I	Liquid 10 mL×1	-20℃
Reagent II	Liquid 0.7 mL×1	2-8℃
Reagent III	Liquid 15 μL×1	2-8℃
Reagent IV	Powder×1	-20℃

Solution Preparation:

1. Reagent II working solution: According to the sample size before use, the working solution was prepared according to the ratio of reagent II: distilled water = 30μL : 70μL (100μL in total, about 5T).
2. Reagent III : The reagent was placed in the EP tube in the reagent bottle and centrifuged to the bottom before use. Before use, 2.5mL distilled water was added to fully dissolve. It can be stored at -20℃ for 4 weeks after subpackage, and repeated freezing and thawing was prohibited (**The liquid may adhere to the wall, and the pipette gun can not be used to absorb 15 μL again, and it can be dissolved by adding distilled water directly.**).
3. Reagent IV : The reagent was placed in the glass bottle in the reagent bottle. Before use, 2.4mL distilled water was added to fully dissolve.It can be stored at -20℃ for 4 weeks after subpackage, and repeated freezing and thawing was prohibited.

Description:

Glycerol-3-phosphate oxidase (GPO, EC 1.1.3.21). GPO is an enzyme present in the cytoplasm, mainly involved in a part of the fat metabolism pathway, known as the glycerol phosphate pathway.

GPO oxidizes glycerol phosphate to dihydroxyacetone phosphate and produces hydrogen peroxide. Under the action of α-glycerophosphate dehydrogenase, NADH and dihydroxyacetone phosphate generate α-glycerophosphate and NAD^+, causing a decrease in absorbance at 340 nm. GPO activity was calculated by measuring the change of NADH.

Reagents and Equipment Required but Not Provided:

Ultraviolet spectrophotometer/microplate reader, water-bath, low temperature centrifuge, adjustable pipette, micro quartz cuvette/96 well UV plate, mortar/ homogenizer/cell ultrasonic crusher, ice and

distilled water.

Operation procedure:

I. Sample preparation

1. Tissue sample: Add extract solution according to the ratio of tissue mass (g) : extract solution (mL) = 1:5 ~10 (it is recommended to weigh 0.1g sample and add 1mL extract solution), after ice bath homogenization, centrifuge at 4℃, 15000g for 10min, take supernatant and placed on the ice for test.

2. Bacteria or cell: The ratio of bacteria/cell amount (10^6) : the volume of extract solution (mL) is 5~10:1(it is suggested to take about 5 million bacteria/cells and add 1 mL of extract solution). Bacteria/cell is split by ultrasonic (placed on ice, 200 W, work time 3 s, interval 7 s, total time 3min). Centrifuge at 15000g for 10 minutes at 4℃, take the supernatant and placed on the ice for test.

3. Serum (plasma) sample: Detect sample directly.

II. Determination procedure

1. Preheat ultraviolet spectrophotometer/microplate reader for more than 30 minutes, adjust wavelength to 340 nm and set ultraviolet spectrophotometer zero with distilled water.
2. Before use, some reagents were preheated at 37℃ for 10 min according to the sample size.
3. Operation table (Add the following reagents to micro quartz cuvette/96 well UV plate)

Reagent name (μL)	Test tube (T)	Blank tube (B)
Sample	50	-
Distilled water	-	50
Reagent I	90	90
Reagent II working solution	20	20
Reagent III	20	20
Reagent IV	20	20

Fully mixed, immediately determine the absorbance A1 at 340 nm for 10 s, and then quickly placed at 37℃ for 10 min to accurately determine the absorbance A2 at 10 min and 10 s (The microplate reader with the temperature control function can set the temperature to 37℃), respectively, recorded as A_{1T}, A_{2T}, A_{1B} ,A_{2B}. Calculate $\Delta A=(A_{1T}-A_{2T})-(A_{1B}-A_{2B})$. The blank tube only need to be measured 1-2 times.

Note : If the number of samples is too large, the ratio of reagent I : reagent II working solution : reagent III : reagent IV = 90 : 20 : 20 : 20 (150 μL, 1 T) can be prepared into working solution to be used.

III. Calculation

1. Using 96-well UV plate to determine :

(1) Calculate by sample protein concentration

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

GPO activity (U/mg prot)=ΔA÷(ε×d)×V_R×10^9÷(Cpr×V_T)÷T×F =107.18×ΔA÷Cpr×F

(2) Calculate by sample mass

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

GPO activity (U/g mass)=ΔA÷(ε×d)×V_R×10^9÷(W÷V_E×V_S)÷T×F =107.18×ΔA÷W×F

(3) Calculate by the number of bacteria or cells

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the consumption of 1 nmol of NADH in the reaction system per minute every 10^6 cells or bacteria.

GPO activity (U/10^6 cell)=ΔA÷(ε×d)×V_R×10^9÷(N÷V_E×V_S)÷T×F =107.18×ΔA÷N×F

(4) Calculate by liquid volume

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the consumption of 1 nmol of NADH in the reaction system per minute every milliliter serum (plasma).

GPO activity (U/mL)=ΔA÷(ε×d)×V_R×10^9÷V_S÷T×F =107.181×ΔA×F

ε: The molar extinction coefficient of NADH, 6.22×10^3L/(mol·cm);

d: 96 well UV plate light path, 0.6cm;

V_R: Reaction system volume, 2×10^{-4}L;

V_T: Sample volume , 0.05mL;

V_E: Extract solution volume, 1mL;

T: Reaction time, 10min;

Cpr: Sample protein concentration, mg/mL;

W: Sample mass, g;

N: Total number of cells or bacteria, count by 10^6;

F: Sample dilution multiple:

10^9: Unit conversion, 1mol=10^9nmol.

2. Using 96-well UV plate to determine :

Change d=0.6 cm in the above formula to d=1 cm (light path of micro quartz cuvette) for calculation.

Note:

1. When A_{1T}<A_{1B} or ΔA>1, the sample can be diluted with distilled water, and the calculation formula can be modified synchronously. When ΔA<0.02, it is recommended to extend the enzymatic reaction time or increase the sample volume (which can reduce the reagent volume at the same time).

Experimental example:

1. 0.113g rat kidney was added to 1 mL extract solution, the supernatant was diluted 2 times with distilled water and operated according to the measurement steps. The ΔA=(A_{1T}-A_{2T})-(A_{1B}-A_{2B})=(0.885-0.628)- (0.606-0.602)=0.253 measured by 96 well UV plate, and

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GPO activity is calculated according to the sample mass:

GPO activity (U/g mass) =107.18×ΔA÷W×F=479.94 U/g mass.

2. Take 50 μL of bovine serum and operated according to the measurement steps. The $\Delta A=(A_{1T}-A_{2T})-(A_{1B}-A_{2B})=(0.771-0.670)-(0.606-0.602)=0.097$ measured by 96 well UV plate, and GPO activity is calculated by liquid volume :

GPO activity (U/mL) =107.18×ΔA×F=10.4 U/mL.

References:

[1] Department of Medical Administration, Ministry of Health of the People 's Republic of China. National Clinical Laboratory Procedures [M].Southeast University Press. 1991.

[2] Wahlefeld, August Wilhelm (1974).Triglycerides Determination after Enzymatic Hydrolysis. 1831–1835.

Related Products:

BC5940/BC5945 Triglyceride(TG) Content Assay Kit(Enzymatic method)

BC6050/BC6055 Glycerol Content Assay Kit

BC/BC Glycerol kinase(GK) Activity Assay Kit

BC6130/BC6135 Glycerol-3-Phosphate(G3P) Content Assay Kit

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