

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: Ultraviolet spectrophotometer

Cat No: BC6120

Size: 50T/48S

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 60 mL×1 | 2-8°C |
| Reagent I | Liquid 30 mL×1 | -20°C |
| Reagent II | Liquid 1.8 mL×1 | 2-8°C |
| Reagent III | Liquid 35 μL×1 | 2-8°C |
| Reagent IV | Powder×1 | -20°C |

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 1T).
2. Reagent III : The reagent was placed in the EP tube in the reagent bottle and centrifuged to the bottom before use. Before use, 6mL distilled water was added to fully dissolve. It can be stored at -20°C for 4 weeks after subpackage, and repeated freezing and thawing was prohibited (**The liquid may adhere to the wall and use the pipette gun to absorb 35 μL again, please directly add distilled water to dissolve**).
3. Reagent IV : The reagent was placed in the glass bottle in the reagent bottle. Before use, 6.6mL distilled water was added to fully dissolve. It can be stored at -20°C 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, water-bath, low temperature centrifuge, adjustable pipette, 1

mL quartz cuvette, 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 1.0mL extract solution), after ice bath homogenization, centrifuge at 4°C, 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°C, take the supernatant and placed on the ice for test.
3. Serum (plasma) sample: Detect sample directly.

II. Determination procedure

1. Preheat ultraviolet spectrophotometer for more than 30 minutes, adjust wavelength to 340 nm and set zero with distilled water.
2. Before use, some reagents were preheated at 37°C for 10 min according to the sample size.
3. Operation table (Add the following reagents to 1mL quartz cuvette)

| Reagent name (μL) | Test tube (T) | Blank tube (B) |
|-----------------------------|---------------|----------------|
| Sample | 250 | - |
| Distilled water | - | 250 |
| Reagent I | 450 | 450 |
| Reagent II working solution | 100 | 100 |
| Reagent III | 100 | 100 |
| Reagent IV | 100 | 100 |

Fully mixed, immediately determine the absorbance A_1 at 340 nm for 10 s, and then quickly placed at 37°C for 10 min to accurately determine the absorbance A_2 at 10 min and 10 s(The microplate reader with the temperature control function can set the temperature to 37°C), 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 = 450 : 100 : 100 : 100 (750 μL, 1 T) can be prepared into working solution to be used.

III. Calculation

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.

$$\text{GPO activity (U/mg prot)} = \Delta A \div (\epsilon \times d) \times V_R \times 10^9 \div (\text{Cpr} \times V_T) \div T \times F = 64.31 \times \Delta A \div \text{Cpr} \times F$$

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.

$$\text{GPO activity (U/g mass)} = \Delta A \div (\epsilon \times d) \times V_R \times 10^9 \div (W \div V_E \times V_S) \div T \times F = 64.31 \times \Delta A \div W \times F$$

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.

$$\text{GPO activity (U/10}^6 \text{ cell)} = \Delta A \div (\epsilon \times d) \times V_R \times 10^9 \div (N \div V_E \times V_S) \div T \times F = 64.31 \times \Delta A \div N \times F$$

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).

$$\text{GPO activity (U/mL)} = \Delta A \div (\epsilon \times d) \times V_R \times 10^9 \div V_S \div T \times F = 64.31 \times \Delta A \times F$$

ϵ : The molar extinction coefficient of NADH, $6.22 \times 10^3 \text{ L}/(\text{mol} \cdot \text{cm})$;

d : 1mL quartz cuvette light path, 1cm;

V_R : Reaction system volume, $1 \times 10^{-3} \text{ L}$;

V_T : Sample volume, 0.25mL;

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, $1 \text{ mol} = 10^9 \text{ nmol}$.

Note:

- When $A_{1T} < A_{1B}$ or $\Delta A > 1$, the sample can be diluted with distilled water, and the calculation formula can be modified synchronously. When $\Delta A < 0.02$, 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:

- 0.113g rat kidney was added to 1 mL extract solution, the supernatant was diluted 4 times with distilled water and operated according to the measurement steps. The $\Delta A = (A_{1T} - A_{2T}) - (A_{1B} - A_{2B}) = (1.441 - 1.127) - (1.027 - 1.011) = 0.298$ measured by 1 mL quartz cuvette, and GPO activity is calculated according to the sample mass:

$$\text{GPO activity (U/g mass)} = 64.31 \times \Delta A \div W \times F = 678.39 \text{ U/g mass.}$$

- Take 250 μL of bovine serum and operated according to the measurement steps. The $\Delta A = (A_{1T} - A_{2T}) - (A_{1B} - A_{2B}) = (1.309 - 1.189) - (1.027 - 1.011) = 0.104$ measured by 1 mL quartz cuvette, and GPO activity is calculated according to the sample mass:

GPO activity (U/mL)=64.31×ΔA×F=6.69 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