Polysaccharide Content 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: BC6130

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℃
Reagent I	Liquid 50 mL×1	2-8℃
Reagent II	Powder×1	-20℃
Reagent III	Liquid 5 mL×1	2-8℃
Reagent IV	Liquid 3 mL×1	2-8℃
Standard	Liquid 0.3 mL×1	2-8℃

Solution Preparation:

1. Reagent II: Reagent II is placed in the EP tube in the reagent bottle. Before use, 4 mL of distilled water was added to fully dissolve, and the unused reagents were stored at -20℃ for 4 weeks. avoid repeated freezing and thawing.
2. Preparation of chromogenic solution : before use, according to the sample size, the ratio of reagent III : reagent IV = 62.5μL : 37.5μL (100μL, about 1T) was prepared and used.
3. Standard: 10 μmol / mL glycerol-3-phosphate .

Description:

Glycerol-3-phosphate (G3P) is an important intermediate in carbohydrate and lipid metabolic pathways and plays an important role in biochemical reactions.

Glycerol-3-phosphate (G3P) generates hydrogen peroxide and dihydroxyacetone phosphate under the catalysis of glycerol phosphate oxidase (GPO), Hydrogen peroxide and 4-AA and 2,4-dichlorophenol produce red products. There is a characteristic absorption peak at 505 nm, according to which the content of G3P can be calculated.

Reagents and Equipment Required but Not Provided:

Spectrophotomete, water-bath, tabletop centrifuge, adjustable pipette, 1 mL glass cuvette, mortar/ homogenizer/cell ultrasonic crusher, ice and distilled water.

Operation procedure:

I. Sample preparation (The sample size to be tested can be adjusted appropriately, and the specific proportion can be used in the literature)

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, boiled in a boiling water bath for 5 min (wrapping the sealing film to prevent the explosion cover), cooled to room temperature and centrifuge at 4℃, 12000rpm for 10min, take the 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). The samples were boiled in a boiling water bath for 5 min (wrapping the sealing film to prevent the explosion cover), cooled to room temperature and centrifuge at 12000g for 10 minutes at 4℃, take the supernatant and placed on the ice for test.
3. Liquid sample : Take 0.3mL liquid sample, add 0.7mL extract solution to boil in a boiling water bath for 5min (wrap the sealing film to prevent the explosion cover), cool to room temperature and centrifuge at 12000g at 4°C for 10min, take the supernatant and place it on ice for testing.

Note :(1) It is recommended to use a spiral port or an EP tube with a lock to react, or to place a small hole on the ordinary EP tube cover to prevent the explosion of the cover ;

(2) If the protein concentration is used to calculate the enzyme activity, part of the homogenate can be retained before boiling for protein content determination.

II. Determination procedure

1. Preheat spectrophotometer for more than 30 minutes, adjust wavelength to 505 nm and set zero with distilled water.
2. Standard preparation: Dilute the standard to 0.5, 0.25, 0.125, 0.0625, 0.03125, 0.015625, 0.0078, 0.0039 μmol/mL with distilled water.
3. Operation table (Add the following reagents to 96 well plate or micro glass cuvette)

Reagent name (μL)	Test tube (T)	Standard tube(S)	Blank tube (B)
Sample	250	-	-
Standard	-	250	-
Distilled water	-	-	250
Reagent I	600	600	600
Accurate reaction at 37°C for 20 min		-	
Reagent II	50	50	50
Chromogenic agent	100	100	100
Mix well and accurate reaction at 37°C for 15 min. The absorbance at 505 nm was measured in a micro glass cuvette / 96-well plate, and recorded as A_T, A_S, A_B. Calculate $\Delta A=A_T-A_B$, $\Delta A_S=A_S-A_B$. The blank tube and standard curve only need to be measured 1-2 times.			

III. Calculation:

1. Standard curve

The standard curve is established according to the concentration of the standard tube (x, μmol/mL) and the absorbance ΔA_S (y, ΔA_S). According to the standard curve, the ΔA_T (y, ΔA_T) is brought into the formula to calculate the sample concentration (x, μmol/mL).

2. Calculate of Polysaccharide Content

(1) Calculate by sample protein concentration

G3P content (μmol/mg prot)=$x \times V_T \div (Cpr \times V_T) \times F = x \div Cpr \times F$

(2) Calculate by sample mass

G3P content (μmol/g mass)=$x \times V_E \div W \times F = x \div W \times F$

(3) Calculate by the number of bacteria or cells

G3P content (μmol/10^6 cell)=$x \times V_E \div N \times F = x \div N \times F$

(4) Calculate by liquid volume

G3P content (μmol/mL) = $3.3 \times x \times F$

V_T: Sample volume , 0.05mL;

V_E: Volume of extract solution added , 1mL;

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;

3.3: The dilution multiple of the liquid sample pretreatment process.

Note:

1. If $\Delta A_T > 1.5$ or $A_T > 2$, the sample can be re-determined after proper dilution with distilled water. If $\Delta A_T < 0.01$, it is recommended that the sample size can be increased or the reaction time of the last step can be prolonged. Note the synchronous modification calculation formula

Experimental example:

1. 0.117g rat livers was added to 1 mL extract solution, the supernatant was operated according to the measurement steps. The $\Delta A_T = A_T - A_B$= 0.298-0.042=0.256 measured by 1 mL glass cuvette. Bring into the standard curve y=2.5148x-0.0091, R^2=0.9996, calculate x=0.105 and G3P is calculated according to the sample mass:

   G3P concent (μmol/g mass)=$x \div W \times F$= 0.897 μmol/g mass

2. .Take 300 μL of rat serum and operated according to the measurement steps. The $\Delta A_T = A_T - A_B$= 0.122-0.052=0.070 measured by 1 mL glass cuvette. Bring into the standard curve y=2.5148x-0.0091, R^2=0.9996, calculate x=0.061 and G3P is calculated according to the sample volume:

   G3P concent (μmol/mL)=$3.3 \times x \times F$=0.201 μmol/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

BC6120/BC6125 Glycerol-3-Phosphate Oxidase Activity Assay Kit

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