Glycogen Assay Kit

Cat#: orb1499910 (manual)

Size: 100T/48S

Microassay

Product composition and storage conditions:

No.	Specifications	Storage Conditions
Extract solution – ES30	100ml ×1	Store at 4 ° C
orb1499910 - A	Powder ×1	Store at 4°C
orb1499910 - B	Powder	Store at 4°C

※Before the formal measurement, be sure to take 2-3 samples with large expected differences for predetermination.

Introduction:

Significance: Glycogen is a high molecular polysaccharide composed of glucose units. It is one of the main storage forms of sugar. It is mainly stored in liver and muscle as standby energy, called liver glycogen and muscle glycogen respectively. Hepatic glycogen regulates blood glucose levels, synthesizes glycogen in the liver when blood glucose is elevated, and breaks down into glucose to supplement blood glucose when blood glucose is decreased. Therefore, hepatic glycogen is important for maintaining the relative balance of blood glucose. Muscle glycogen is the storage form of sugar in the muscle. When intensive exercise consumes a large amount of blood glucose, muscle glycogen cannot be directly decomposed into blood glucose, but must be decomposed to produce lactic acid, circulates to the liver with blood, and is converted into hepatic glycogen or glucose through gluconeogenesis.

Principle: Anthrone method. Glycogen was extracted with strong alkaline extract, and the content of glycogen was determined with anthrone chromogenic agent under strong acid condition.

Own supplies:

Visible spectrophotometer/microplate reader, water bath, adjustable pipettes, microcuvette/96-well plates, concentrated sulfuric acid (not allowed for express delivery) and distilled water..

Glycogen extraction:

1. Cells or bacteria: Collect 5~10 million bacteria or cells into the centrifuge tube, and discard the supernatant after centrifugation; Add 0.75mL Extract solution ES30 and break down bacteria or cells by ultrasonic wave (power 20% or 200 W, ultrasonic wave 3s, interval 10 s, repeat 30times); Transfer to 10mL test tube, boil in the water bath for 20 min (cover tightly to prevent water loss), shake the tube once every 5 min, and mix well; take out the test tube and cool it down, make volume up to 5ml with distilled water, mix well, centrifuge for 10 min at 8000 g 25°C, and take the supernatant for test.

2. Tissue: Weigh 0.1~0.2 g sample, add 0.75 ml Extract solution ES30 and fully homogenate; Transfer to 10 ml tube; 95℃ Water bath 20 min (cover tightly to prevent water loss), shake the tube once every 5 min, and mix well; After all the tissues are dissolved, take out the test tube and cool it down, make volume up to 5ml with distilled water, mix well, centrifuge for 10 min at 8000 g 25℃, and take the supernatant for test.

Measurement steps:

1. Preheat the visible spectrophotometer/microplate reader for at least 30 minutes, adjust the wavelength to 620nm, adjust to zero with distilled water.
2. Adjust the water bath to 100 ° C.
3. Preparation of orb1499910 -A working solution: Add 1 mL of distilled water into orb1499910 -A, which is the 10 mg/mL glucose solution, and then dilute the solution to 0.1 mg/mL with distilled water for use.
4. Preparation of orb1499910 -B working solution: Add 6mL distilled water into orb1499910 -B, slowly pour 24mL concentrated sulfuric acid, fully dissolve and mix well before use; The remaining reagents can be stored at 4 °C for one week.
5. Sample table (reaction in EP tube):

Reagent name	Blank tube (ul)	Standard tube (ul)	Measuring tube (ul)
Samples			60
orb1499910 - A		60	
Distilled water	60		
orb1499910 - B	240	240	240
Mix well, boil in the water bath for 10 min (cover tightly to prevent water loss), cool it down, take 200 μL and transfer to the micro-cuvette or 96-well plate, read the absorbance of blank tube, standard tube and measuring tube at the wavelength of 620nm, and record as A1,A2and A3 respectively.			

Note:

1. The blank tube and standard tube are tested only for 1-2 times.

2. If A3-A1 is greater than two, dilute the sample with distilled water and multiply by the corresponding dilution factor.

Calculation formula:

1. Calculated by sample weight

Glycogen (mg/g fresh weight) = (C standard ×V1) × (A3-A1) ÷ (A2-A1) ÷ (W×V1÷V2) ÷1.11

= 0.45×(A3-A1) ÷ (A2-A1) ÷ W

2. Calculated by sample protein concentration

Glycogen (mg/mg prot) = (C standard ×V1) × (A3-A1) ÷ (A2-A1) ÷ (V1×Cpr) ÷1.11

= 0.09×(A3-A1) ÷ (A2-A1) ÷ Cpr

3. Calculated by number of bacteria or cells

Glycogen (mg/10^4 cell) = (C standard ×V1)× (A3-A1) ÷ (A2-A1) ÷ (number of bacteria or cells ×V1÷V2) ÷ 1.11

= 0.45× (A3-A1) ÷ (A2-A1) ÷ number of bacteria or cells

In the calculation formula: 1.11: It is the constant that the glucose content measured by this method is converted to glycogen content, that is, the color of 111ug glucose with anthrone reagent is equivalent to that of 100ug glycogen with anthrone reagent; C Standard tube: Standard tube concentration, 0.1 mg/mL; V1: Volume of glycogen extract added to the reaction system, 0.06mL; V2: Volume of extract added, 5 mL; Cpr: Sample protein concentration, mg/mL; W: Sample fresh weight, g; Number of bacteria or cells: 10^4 (10000) .