Glycogen Assay Kit

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

Operation Equipment: Spectrophotometer/ microplate reader

Catalog Number: AK0290-100T-96S

Components:

Extract reagent: 100mL×1, storage at 4℃.

Reagent I: Powder×1, storage at 4℃.10 mg of glucose, add 1 mL of distilled water to dissolve it before use. The reagent can be stored at 4℃ for two weeks.

Reagent II: Powder×2, storage at 4℃.

Working solution: Before use, take 1 bottle of reagent II and pour it into 3 mL of distilled water, slowly pour in 12 mL of concentrated sulfuric acid, fully dissolve and mix well before use. Unused reagents can be stored at 4°C for a week

Product Description

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 the liver and muscle as backup energy, and is called liver glycogen and muscle glycogen, respectively. Glycogen can regulate blood glucose concentration. Glycogen can be synthesized in the liver when blood glucose rises. When blood sugar decreases, liver glycogen is broken down into glucose to supplement blood sugar. Therefore, liver glycogen is important to maintain the relative balance of blood sugar. Muscle glycogen is a form of glycogen storage in muscles. When lots of blood sugar is consumed during strenuous exercise, muscle glycogen cannot be broken down directly into blood sugar. It must first be broken down to produce lactic acid, which is circulated to the liver with the blood, and transformed into liver glycogen through glycogen glucose.

Determination principle: anthrone method. Glycogen is extracted with strong alkaline extract, and the glycogen content is measured using an anthrone method under strongly acidic conditions.

Reagents and Equipment Required but Not Provided.

Spectrophotometer/microplate reader, water bath,desk centrifuge, transferpettor, micro glass cuvette/96-well plate, concentrated sulfuric acid (H_2SO_4) and distilled water.

Procedure:

I. Sample extraction:

1、Cells or bacteria: Collect 5-10 million bacteria or cells into a centrifuge tube, discard the supernatant after centrifugation; add 0.75mL of extraction reagent to ultrasonically break bacteria or cells (power 200W, ultrasonic 3s, 10s interval, repeat 30 times)); Transfer to a 10mL tube, boil in a boiling water bath for 20min (close tightly to prevent water loss), shake the tube every 5min to fully mix; take out the tube and cool, take up to 5mL with distilled water and mix. Centrifuge at 8000g and 25℃ for

10min, take the supernatant for testing.

2、Tissue: Weigh 0.1~0.2g sample, put it in a 10 mL tube and cut as much as possible with surgical scissors, add 0.75 mL extraction solution, boil in boiling water bath for 20min (close tightly to prevent water loss), shake the test tube every 5min to fully mix. After all the tissue dissolved, take out the tube and cool down, then make up to 5mL with distilled water.

II. Determination procedure:

1. Preheat the **s**pectrophotometer/ microplate reader for 30 min, adjust wavelength to 620 nm, the **s**pectrophotometer set zero with distilled water.
2. Reagent Idilution: Take 10 μL of 10 mg/mL glucose standard solution, add 990 μL of distilled water, mix well, and prepare a 0.1 mg/mL glucose solution for later use. (In the experiment, each tube needs 60 μL, in order to reduce the experimental error, a large volume is prepared.)
3. Sampling table (add the following reagents in EP tube)

Reagent(μL)	Blank Tube (A1)	Standard Tube (A2)	Test Tube (A3)
Sample			60
Reagent I		60	
distilled water	60		
Reagent II	240	240	240

Mix well, place in a boiling water bath for 10 minutes (close tightly to prevent water loss), cool, and take 200 μL into micro glass cuvette/96-well plate to read the absorbance of the blank tube, standard tube, and measurement tube at 620 nm, and record them as A1, A2, and A3. The blank tube and standard tube need only be tested once or twice.

III. Calculation:

1. Sample weight

Sorbitol (mg/g fresh weight) =(Cs×V1)×(A3-A1)÷(A2-A1)÷(W×V1÷V2)÷1.11

= 0.450×(A3-A1)÷（A2-A1)÷W

2. Protein concentration

Sorbitol (mg/mg prot) = (Cs×V1)×(A3-A1) ÷(A2-A1) ÷(V1×Cpr) ÷1.11

=0.09×(A3-A1) ÷（A2-A1) ÷Cpr

3. The number of bacteria or cells:

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

= 0.450×(A3-A1)÷（A2-A1)÷number of bacteria or cells

1.11: It is a constant that glucose content converted to glycogen content, That is, the color of 111 μg of glucose with anthrone reagent is equivalent to that of 100 μg of glycogen with anthrone reagent.

Cs: the concentration of standard, 0.1mg/mL

V1: sample volume, 0.06 mL;

V2: Total sample volume, 5 mL;

Cpr: sample protein concentration, mg/mL;

W: Sample weight, g

Number of bacteria or cells: 10^4 as the unit, ten thousand

Note:

If A is greater than 1.4, dilute the sample with distilled water and multiply it by the corresponding dilution factor in the calculation formula.

Recent Produt Citations：

[1] Zheng J, Yu J, Jia M, et al. Indole enhances the survival of Pantoea ananatis YJ76 in face of starvation conditions[J]. Journal of basic microbiology, 2017, 57(7): 633-639.

[2] Xu L, Li Y, Yin L, et al. miR-125a-5p ameliorates hepatic glycolipid metabolism disorder in type 2 diabetes mellitus through targeting of STAT3[J]. Theranostics, 2018, 8(20): 5593.

[3] Ce Gu,Panpan Li,Wei Liu,et al. The role of insulin in transdifferentiated hepatocyte proliferation and function in serum-free medium. Journal of Cellular and Molecular Medicine. April 2019;(IF4.658)

References：

[1] Raunkjær K, Hvitved-Jacobsen T, Nielsen P H. Measurement of pools of protein, carbohydrate and lipid in domestic wastewater[J]. Water research, 1994, 28(2): 251-262.

[2] Carroll N V, Longley R W, Roe J H. The determination of glycogen in liver and muscle by use of anthrone reagent[J]. J biol Chem, 1956, 220(2): 583-593.

Technical Specification:

The detection limit: 0.002 mg/mL

The linear range: 0.003125-0.25 mg/mL