Glycogen Assay Kit

Cat #: orb1499911 (manual)

Visible Spectrophotometer

Size: 50T/48S

Product composition and storage conditions :

No.	Specifications	Storage conditions
Extraction solution-ES30	Liquid×1 bottle	Store at 4℃
orb1499911-A	Powder×1 bottle	Store at 4℃
orb1499911-B	Powder×1 bottle	Store at 4℃

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

Introduction

Significance: Glycogen is a macromolecular polysaccharide composed of glucose units, which is one of the main storage forms of sugar. Glycogen is mainly stored in the liver and muscle as spare energy, called liver Glycogen and muscle Glycogen, respectively. Liver glycogen can regulate blood glucose concentration, when blood glucose increased in the liver, synthesis of glycogen, blood glucose decreased, liver glycogen principle decomposed into glucose to supplement blood glucose. Therefore, liver glycogen is very important to maintain the relative balance of blood glucose. Muscle glycogen is the storage form of sugar in the muscle. When intense exercise consumes a large amount of blood sugar, muscle glycogen can not be directly broken down into blood sugar. It must first be broken down to produce lactic acid, which circulates through the blood to the liver, change to liver glycogen or glucose by gluconeogenesis.

Principle: Anthrone method. Glycogen was extracted from strong alkaline extract, and the content of glycogen was determined by anthrone color reagent under strong acid condition.

Own suppliers: Visible photometer, water bath, Centrifuge, adjustable pipette, 1 ml glass colorimeter, concentrated sulfuric acid and double-distilled water.

Glycogen extraction:

1. Cell or bacterium: Collect 5 million ~ 10 million bacteria or cells into a centrifuge tube, centrifuge and then discard the supernatant, add 0.75 ml extraction solution-ES30 and then break the bacteria or cells by ultrasonic wave (power 20% or 200 W, ultrasonic wave 3 s, interval 10 s, repeat 30 times). Transfer to 10 ml tube, 95 ° C water bath for 20 min (cover tightly to prevent water loss) , shake the tube once every 5 min to make full mixing, take out the tube after cooling, with distilled water to 5 ml, mixing, 8000 g 25 ° C centrifuge for 10 min, take the supernatant for testing.
2. Tissue：Weigh 0.1 ~ 0.2 g sample, add 0.75 ml extraction solution-ES30 to fully homogenize, transfer to 10 ml test tube, 95 ° C water bath for 20 min (tightly cover to prevent water loss) , shake test tube once every 5 min to fully mix; After the whole tissue was dissolved, take out the tube after cooling, with distilled water to 5 ml, mixing, 8000 g 25 ° C centrifuge for 10 min, take the supernatant for testing.

Measurement operation:

1. Preheat the photometer for at least 30 minutes, adjust the wavelength to 620 nm, and zero with the distilled water.
2. Adjust water bath to 100 ° C.
3. Preparation of orb1499911-A working solution: Add 1ml distilled water into orb1499911-A, that is 10 mg/mL glucose solution, and then dilute with distilled water to 0.1 mg/mL working solution. The solution could be stored at 4 ° C for a week.
4. Preparation of orb1499911-B working solution: Add 10 ml of distilled water into orb1499911-B, and then slowly add 40 ml of concentrated sulfuric acid, fully dissolve and mix; The rest of the reagent could be stored at 4 ° C for a week.
5. Sampling Table (reaction in EP tube) :

Name of reagent	Blank tube (ul)	Standard tube (ul)	Measuring tube (ul)
Samples to be tested			250
orb1499911-A		250	
Distilled water	250		
orb1499911-B	1000	1000	1000
After mixing, boiling water bath for 10 min (cover tightly to prevent water loss) , cooling, zero at 620 nm wavelength with distilled water, read the absorbance of blank tube, standard tube and measuring tube, and record as a 1, a 2 and a 3 respectively.			

Note: the blank tube and standard tube only need to test 1-2 times.

Calculation formula:

1. Calculate according to the 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

3. Calculate according to the protein content

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 the number of bacteria or cells

Glycogen (mg/104 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 ： is the constant from which the glucose content measured by this method is converted to the glycogen content, means that 111 ug of glucose with Anthrone reagent is equivalent to 100 ug of glycogen with Anthrone reagent; C-Standard tube：Standard tube concentration, 0.1mg/mL； V1：The volume of glycogen extract was added into the reaction system, 0.25mL； V2：The volume of the extraction liquid adding ,5mL； Cpr: Sample protein concentration, mg/mL； W：The fresh weight of the sample, g； Number of bacteria or cells：in units of 10^4.

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