Glycogen Content Assay Kit (Anthrone, Micro Method)

Cat. No.: G1501748 | Pack size: 96T | Storage: Store at 2-8°C,Protected from light

Product Information

Item	Description
Specifications & Purity	BioReagent
Application	Cell Metabolism
Sensitivity	Light-sensitive
Storage Conditions	Store at 2-8°C,Protected from light
Shipped In	Wet ice
Stability And Storage	Store at 2-8°C long term (12 months). Store in the dark.

Product Description

Glycogen is a macromolecular polysaccharide composed of glucose and serves as one of the primary storage forms of sugar. It is mainly stored in the liver and muscles as reserve energy, referred to as liver glycogen and muscle glycogen, respectively. Liver glycogen regulates blood glucose concentration; when blood sugar rises, glycogen can be synthesized in the liver, and when blood sugar decreases, liver glycogen is broken down into glucose to supplement blood sugar. Therefore, liver glycogen is crucial for maintaining the relative balance of blood glucose. Muscle glycogen is the storage form of sugar in muscles. During strenuous exercise that consumes large amounts of blood sugar, muscle glycogen cannot be directly broken down into blood sugar but must first decompose to produce lactic acid, which circulates to the liver via the bloodstream and is converted into liver glycogen and glucose through gluconeogenesis.

Detection Principle: Glycogen is extracted using a strong alkaline extraction buffer. Under strong acidic conditions, it forms a blue compound with the anthrone chromogen, which has a characteristic absorption peak at 620 nm. Within a certain concentration range, the glycogen content is linearly

related to the absorbance at 620 nm. The glycogen content in the sample can be calculated based on the standard curve.

Detection Range: 0.003125 - 0.25 mg/mL.

Sensitivity: 0.003125 mg/mL.

Applicable Samples: Animal tissues, bacteria, cells.

Contents & Storage

G1501748	Component	96T	Storage
G1501748A	Extraction Buffer	120 mL	2-8°C
G1501748B	Chromogen	1EA	2-8°C. Store in the dark.
G1501748C	Standard	1 mL	2-8°C

Note: It is recommended to perform preliminary experiments using 2-3 samples expected to have significant differences before formal testing.

User-Provided Instruments and Consumables

1.Microplate reader or visible spectrophotometer (capable of measuring absorbance at 620 nm).

2.Low-temperature centrifuge, Water bath.

3.96-well plate or micro glass cuvettes, Adjustable pipettes and tips, EP tubes.

4.Deionized water, Concentrated sulfuric acid.

Experimental Procedure

1. Reagent Preparation

Reagent Name	Reagent Preparation	Precautions
Extraction Buffer	Ready-to-use; equilibrate to room temperature before use.	Store at 4°C. Corrosive; please take protective measures during handling.
Chromogen	First, dissolve the powder in 7.2 mL of deionized water. Then slowly add 28.8 mL of concentrated sulfuric acid. Mix thoroughly after complete dissolution.	Store at 4°C protected from light; valid for one week. Toxic; please take protective measures during handling.
Standard	-	Store at 4°C.

2. Standard Curve Setup

Dilute the 1 mg/mL standard with deionized water to prepare standard solutions of 0.25, 0.1, 0.05, 0.025, 0.0125, 0.00625, and 0.003125 mg/mL as shown in the table below.

No.	Standard Volume Std.1 Std.2	Deionized Water Volume (µL) 100µL of 1mg/mL 300 160µL of Std.1 240	Concentration (mg/mL) 0.25 0.1
Std.3	200µL of Std.2	200	0.05
Std.4	200µL of Std.3	200	0.025
Std.5	200µL of Std.4	200	0.0125
Std.6	200µL of Std.5	200	0.00625
Std.7	200µL of Std.6	200	0.003125

Note: A standard curve must be prepared for each experiment. Diluted standard solutions are unstable and must be used within 4 hours.

3. Sample Preparation

Note: Fresh samples are recommended. If not used immediately, samples can be stored at -80°C for up to 1 month.

3.1 Tissues

Weigh 0.1 g of tissue and place it in a 10 mL test tube. Add 0.75 mL of Extraction Buffer. Boil in a water bath for 20 minutes (stopper the tube tightly to prevent water evaporation). Shake the tube every 5 minutes to mix thoroughly. After the tissue is completely dissolved, remove the tube and let it cool. Dilute to 5 mL with deionized water, mix well. Centrifuge at 8,000 g, 25°C for 10 minutes. Collect the supernatant for detection.

3.2 Cells or Bacteria

Collect 5 million bacteria or cells into an EP tube. Centrifuge and discard the supernatant. Add 0.75 mL of Extraction Buffer and disrupt the bacteria or cells by ultrasonication (power 200 W, ultrasonicate for 3 s, interval 10 s, repeat 30 times). Transfer to a 10 mL test tube. Boil in a water bath for 20 minutes (stopper the tube tightly to prevent water evaporation). Shake the tube every 5 minutes to mix thoroughly. Remove the tube and let it cool. Dilute to 5 mL with deionized water, mix well. Centrifuge at 8,000 g, 25°C for 10 minutes. Collect the supernatant for detection.

Note: For protein concentration determination, Aladdin BCA Protein Quantification Kit (B665595) or Ready-to-Use BCA Protein Quantification Kit (R1491648) are recommended.

4. Assay Steps

4.1 Instrument Preparation: Preheat the microplate reader or visible spectrophotometer for at least 30 minutes. Set the wavelength to 620 nm. For visible spectrophotometers, zero the instrument with deionized water.

4.2 Sample Assay: Add reagents sequentially to EP tubes as follows:

Reagent	Blank Tube (μL)	Standard Tube (μL)	Test Tube (μL)
Sample	0	0	60
Standard	0	60	0
Deionized Water	60	0	0
Chromogen	240	240	240

4.3 Mix well. Incubate in a 95°C water bath for 10 minutes (cap tightly to prevent evaporation). Cool. Transfer 200 μL to a 96-well plate or micro glass cuvette. Measure the absorbance at 620 nm, recorded as A blank, A standard, and A test. Calculate ΔA test = A test - A blank and ΔA standard = A standard - A blank. Note: It is recommended to perform preliminary experiments with 2-3 samples expected to have significant differences before formal testing. If ΔA test is less than 0.001, appropriately increase the sample amount. If ΔA test is greater than 1.5, dilute the sample further with deionized water (multiply the result by the dilution factor) or reduce the amount of sample used for extraction.

5. Result Calculation

Note: We provide both derived and simplified calculation formulas, which are equivalent. The simplified formulas in bold are recommended as the final calculation formulas.

5.1 Standard Curve Plotting Plot the standard curve with standard concentration as the y-axis and ΔA standard as the x-axis (using concentration as the y-axis facilitates calculation). Substitute ΔA test into x to calculate y (mg/mL).

5.2 Sample Glycogen Content Calculation

(1) Based on sample mass:

Glycogen (mg/g) = (y × Vsample) ÷ (W × Vsample ÷ Vtotal) ÷ 1.11 × n = 4.5 × y ÷ W × n

(2) Based on sample protein concentration:

Glycogen (mg/mg prot) = (y × Vsample) ÷ (Vsample × Cpr) ÷ 1.11 × n = 4.5 × y ÷ Cpr × n

(3) Based on bacterial or cell count:

Glycogen (mg/10^4) = (y × Vsample) ÷ (N × Vsample ÷ Vtotal) ÷ 1.11 × n = 4.5 × y ÷ N × n

Parameter Description:

1.11: Constant for converting glucose content measured by this method to glycogen content (i.e., 100 μg glucose color developed with anthrone reagent is equivalent to that of 111 μg glycogen).

V sample: Volume of test sample added to the reaction system, 0.06 mL.

W: Sample mass, g.

V total: Total volume of the sample extract, 5 mL.

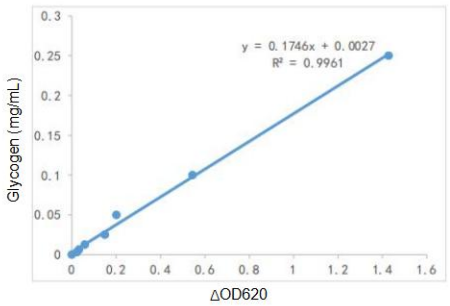
n: Dilution factor. Cpr: Sample protein concentration, mg/mL.

Bacterial or Cell Count: In units of 10^4 (ten thousands)

6. Result Presentation

Typical Standard Curve: y = 0.1746x + 0.0027, R^2 = 0.9961

(The following data and curve are for reference only; users must establish their own standard curve based on their experiment.



Precautions

1. It is recommended to perform preliminary experiments using 2-3 samples expected to have significant differences before formal testing.

2. This product is for scientific research use only and is not intended for clinical diagnosis. For your safet y and health, please wear a lab coat and disposable gloves during operation.

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