

Glycogen Content Assay Kit (Anthrone, Micro-method)

G1522862

Storage 4°C. Protect from light.

Shipping Shipped with ice packs. Please store under the specified storage conditions immediately upon receipt.

Product Introduction

Glycogen is a high-molecular-weight polysaccharide composed of glucose units and is one of the main storage forms of glucose. 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 glucose rises, it is synthesized into glycogen in the liver; when blood glucose decreases, liver glycogen is broken down into glucose to supplement blood glucose. Therefore, liver glycogen is very important for maintaining the relative balance of blood glucose. Muscle glycogen is the storage form of glucose in muscles. During strenuous exercise that consumes a large amount of blood glucose, muscle glycogen cannot be directly broken down into blood glucose; it must first be broken down into lactate, which is transported through the bloodstream to the liver, where it is converted into liver glycogen and glucose via gluconeogenesis.

The detection principle of this kit is as follows: Glycogen is extracted using a strong alkaline extraction buffer. Under strong acidic conditions, it reacts with the anthrone chromogenic reagent to form a blue compound with a characteristic absorption peak at 620 nm. Within a certain concentration range, the glycogen content is linearly related to the absorbance at 620 nm. Based on the standard curve, the glycogen content in the sample can be calculated.

Components

G1522862	Components	Appearance	100 T	Storage	Quantity Per Test
G1522862A	Alkaline Extraction Buffer	Liquid	100 mL	4°C	0.75 mL
G1522862B	Chromogenic Reagent	Powder	4 tubes	4°C, Store in the dark.	200 µL
G1522862C	Glucose Standard (1 mg/mL)	Liquid	2 mL	4°C, Store in the dark.	100 µL

Usage Protocol

1. Reagent Preparation

- 1) Equilibrate the Alkaline Extraction Buffer to room temperature. This reagent is corrosive; please take appropriate protective measures during handling.

Web: <https://www.aladdinsci.com>

- 2) Take one vial of Chromogenic Reagent, add 5 mL of concentrated sulfuric acid, dissolve thoroughly, and store protected from light. This solution is valid for 3 days at 4 °C; use it as soon as possible after preparation..

2. Standard Curve Setup

Dilute the 1 mg/mL standard with deionized water according to the table below to prepare standard solutions of 0.25, 0.1, 0.05, 0.025, 0.0125, 0.00625, and 0.003125 mg/mL. The glucose standard is provided sterile. Once opened, storage at 4 °C may lead to contamination. For first use, open it in a clean bench and dispense aseptically before storing at 4 °C. If sterility cannot be guaranteed, aliquot and freeze at –20 °C.

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

3. Sample Preparation

Note: Fresh samples are recommended. If the experiment is not performed immediately, samples can be stored at –80 °C for one month.

1) Tissue Samples

Weigh 0.1 g of sample, place it into a 10 mL test tube, add 0.75 mL of Alkaline Extraction Buffer, and boil in a boiling water bath for 20 min (cap the test tube tightly to prevent water evaporation). Shake the tube once every 5 min to mix thoroughly. After the tissue is completely dissolved, remove the tube, cool it, and add deionized water to a final volume of 5 mL. Mix well, centrifuge at 8,000 × g for 10 min at 25 °C, and collect the supernatant for testing.

2) Cell or Bacterial Samples

Collect 5 × 10⁶ bacteria or cells into an EP tube, wash with 1× PBS, centrifuge, and discard the supernatant. Add 0.75 mL of Alkaline Extraction Buffer and disrupt the bacteria or cells by ultrasonication (power 200 W, sonicate for 3 s, pause for 10 s, repeat 30 times). Transfer the mixture to a 10 mL test tube, boil in a boiling water bath for 20 min (cap the test tube tightly to prevent water evaporation), shaking once every 5 min to mix thoroughly. Remove the tube, cool it, and add deionized water to a final volume of 5 mL. Mix well, centrifuge at 8,000 × g for 10 min at 25 °C, and collect the supernatant for testing.

4. Sample Detection

Add the following reagents into an EP tube:

Reagent	Blank tube (μL)	Standard tube (μL)	Sample tube (μL)
Sample	0	0	100
Standard solution	0	100	0
Deionized water	100	0	0
Chromogenic reagent	200	200	200

Mix well, then react in a boiling water bath for 5 min (cap tightly to prevent water evaporation). Cool under running water, then transfer 200 μL to a 96-well plate. Measure the absorbance at 620 nm, recorded as A_{blank} , A_{standard} , and A_{sample} respectively. Calculate $\Delta A_{\text{sample}} = A_{\text{sample}} - A_{\text{blank}}$, $\Delta A_{\text{standard}} = A_{\text{standard}} - A_{\text{blank}}$.

Note: It is recommended to perform a preliminary experiment using 2-3 samples with expected large differences before formal testing. If ΔA_{sample} is less than 0.001, the sample volume can be appropriately increased. If ΔA_{sample} exceeds the range of the standard curve, the sample can be further diluted with deionized water and the result multiplied by the dilution factor, or the amount of sample used for extraction can be reduced.

5. Result Calculation

Plot the standard curve with the standard concentration as the X-axis and $\Delta A_{\text{standard}}$ as the Y-axis. Substitute ΔA_{sample} into Y to obtain X (mg/mL).

Total glucose content in the extract (mg) = $X \times V_{\text{sample}} \times (V_{\text{total}} \div V_{\text{sample}}) \times n = X \times V_{\text{total}} \times n$

Total glycogen content in the extract (mg) = $X \times n \times V_{\text{total}} \div 1.11$

1) Calculated by sample mass:

Glycogen (mg/g) = $(X \times n \times V_{\text{total}} \div 1.11) \div W$

2) Calculated by sample protein concentration:

Glycogen (mg/mg prot) = $(X \times n \times V_{\text{total}} \div 1.11) \div (C_{\text{pr}} \times V_{\text{total}})$

3) Calculated by bacterial or cell number:

Glycogen (mg/10⁴ cells) = $(X \times n \times V_{\text{total}} \div 1.11) \div N$

Parameter description:

1.11: constant for converting glucose content measured by this method to glycogen content, i.e., the color intensity of 100 μg glycogen with anthrone reagent is equivalent to that of 111 μg glucose with anthrone reagent;

V_{sample} : volume of the test sample added to the reaction system, 0.1 mL;

W: sample mass, g;

V_{total} : total sample volume, 5 mL;

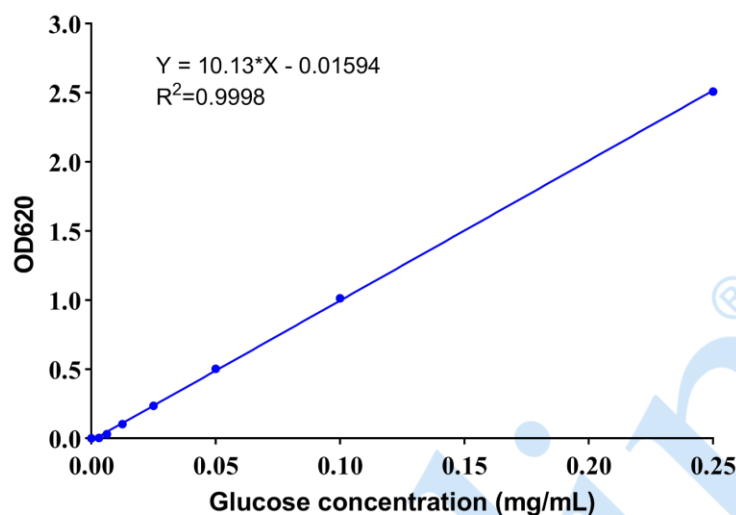
n: dilution factor;

Cpr: protein concentration of the sample, mg/mL;

N: number of bacteria or cells, in units of 10^4 .

6. Results Presentation

Standard curve:



Precautions

1. Before formal testing, it is recommended to perform a preliminary experiment using 2-3 samples with expected large differences.
2. The concentration of concentrated sulfuric acid must be 95%-98% analytical grade and the bottle should not have been opened for too long.
3. When preparing the chromogenic reagent, first transfer the powder into a glass container, then add a small amount of concentrated sulfuric acid, stir carefully to dissolve, then add the remaining concentrated sulfuric acid. The prepared chromogenic reagent can be stored at 4 °C, but for no more than 3 days. It is recommended to prepare fresh immediately before use.
4. The glucose standard is provided sterile. Once opened, storage at 4 °C may lead to contamination. For first use, open it in a clean bench and dispense aseptically before storing at 4 °C. If sterility cannot be guaranteed, aliquot and freeze at -20 °C.
5. This product is for research use only and is not suitable for clinical diagnosis. For your safety and health, please wear a lab coat and disposable gloves when handling.