Glycogen Assay Kit (Colorimetric)

Cat #: orb1173189 (manual)

Product name: Glycogen Assay Kit (Colorimetric)

Catalog number: orb1173189

Detection range: 0.003125-0.25 mg/mL

Applicable samples: Animal Tissues, Bacteria, Cells

Storage: Stored at 4℃ for 12 months, protected from light

Assay Principle

Glycogen is a polymer polysaccharide composed of glucose. It is one of the main storage forms of glucose. It is mainly stored in liver and muscle as reserve energy, which is called liver glycogen and muscle glycogen, respectively. Liver glycogen can regulate the concentration of blood glucose. When blood glucose rises, glycogen can be synthesized in the liver. When blood glucose decreases, liver glucose principle decomposes 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 glucose in muscle. When strenuous exercise consumes a large amount of blood glucose, muscle glycogen cannot be decomposed directly into blood glucose. It was decomposed to produce lactic acid and circulate to the liver with blood. It is transformed into liver glycogen and glucose by gluconeogenesis. CheKine™ Micro Glycogen Assay Kit can detect glycogen concentration in animal tissues, bacteria and cells. The principle of kit is strong alkaline extract glycogen. under strong acidic conditions, blue compounds with anthrone chromogenic agent with characteristic absorption peak at 620 nm. In a certain concentration range, glycogen concentration is linearly related to 620 nm absorbance. According to the standard curve, the glycogen concentration in the sample can be calculated.

Materials Supplied and Storage Conditions

Kit components	Size 96 T	Storage conditions
Extraction Buffer	120 mL	4℃
Chromogen	Powder×1 vial	4℃, protected from light
Standard	1 mL	4℃

Note: Before formal testing, it is recommended to select 2-3 samples with large expected differences for pre-experiment.

Materials Required but Not Supplied

- Microplate reader or visible spectrophotometer capable of measuring absorbance at 620 nm
- Refrigerated centrifuge, water bath
- 96-well plate or microglass cuvette, precision pipettes, disposable pipette tips, EP tube
- Deionized water, concentrated sulfuric acid

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Reagent Preparation

Extraction Buffer: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃.

Chromogen: Add 7.2 mL deionized water to dissolve the powder, then slowly pour into 28.8 mL concentrated sulfuric acid, fully

dissolve and mix. 4℃ avoid light for one week.

Note: Extraction Buffer is corrosive, Chromogen is toxic. Please take protective measures when operating.

Standard: 1 mg/mL. Store at 4℃.

Standard curve setting: Dilute 1 mg/mL standard with deionized water to 0.25, 0.1, 0.05, 0.025, 0.0125, 0.00625, 0.003125 mg/mL standard solution as shown in the table below.

NUM.	Volume of Standard	Deionized Water (μL)	Concentration (mg/mL)
Std.1	100 μL 1 mg/mL	300	0.25
Std.2	160 μL of Std.1	240	0.1
Std.3	200 μL of Std.2	200	0.05
Std.4	200 μL of Std.3	200	0.025
Std.5	200 μL of Std.4	200	0.0125
Std.6	200 μL of Std.5	200	0.00625
Std.7	200 μL of Std.6	200	0.003125

Notes: Always prepare fresh standards per use; Diluted Standard Solution is unstable and must be used within 4 h.

Sample Preparation

Note: Fresh samples are recommended. If the experiment is not carried out immediately, the samples can be stored at -80℃ for 1 month.

1. Tissue samples: Weigh 0.1 g tissue, put it in 10 mL test tube, add 0.75 mL Extraction Buffer, boil 20 min (cover tightly to prevent water loss). shake test tube every 5 min, mix well. When the tissue is dissolved, dilute with deionized water to 5 mL, centrifuge at 8,000 g for 10 min at 25°C. Use supernatant for assay.
2. Bacteria or Cells: collect 5 million bacteria or cells into EP tubes, wash with PBS, add 0.75 mL Extraction Buffer, Ultrasonic lysis of bacteria or cells (200 W, work 3 s, intermittent 10 s, work 30 times). Transferred to 10 mL tube, boil 20 min (cover tightly to prevent water loss), shake the tube every 5 min, mix well. Dilute with deionized water to 5 mL, mix well, 8,000 g, 25°C centrifuge 10 min. Use supernatant for assay.

Note: If the protein concentration of the sample is need to determined, it is recommended to use Biorbyt Protein Quantification Kit (BCA Assay) to measure the protein concentration of the sample.

Assay Procedure

1. Preheat the microplate reader or visible spectrophotometer for more than 30 min, and adjust the wavelength to 620 nm. Visible spectrophotometer was returned to zero with deionized water.
2. Add the following reagents respectively into each EP tube:

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

3. Mix well, incubate in 95℃ 10 min (Cover tightly to prevent moisture evaporation), add 200 μL of the reaction mix to 96-well plate or microglass cuvette. Measure absorbance at OD620 nm and record as A_{Blank}, $A_{Standard}$, A_{Test}. Calculate $\Delta A_{Test}=A_{Test}-A_{Blank}$, $\Delta A_{Standard}=A_{Standard}-A_{Blank}$.

Note: In order to guarantee the accuracy of experimental results, need to do a pre-experiment with 2-3 samples. If ΔA_{Test} is less than 0.001, increase the sample quantity appropriately. If ΔA_{Test} is greater than 1.5, the sample can be appropriately diluted with deionized water, the calculated result multiplied by the dilution factor, or decrease the sample quantity appropriately.

Data Analysis

Note: We provide you with calculation formulae, including the derivation process and final formula. The two are exactly equal. It is suggested that the concise calculation formula in bold is final formula.

1. Drawing of standard curve:

With the concentration of the standard solution as the y axis and the $\Delta A_{Standard}$ as the x axis, draw the standard curve. Substitute the ΔA_{Test} into the equation to obtain the y value (mg/mL).

2. Calculated by sample quality

Glycogen (mg/g)$=1.11\times(y\times V_{Sample})\div(W\times V_{Sample}\div V_{Total\ sample})\times n$**$=5.55\times y\div W\times n$**

3. Calculated by protein concentration

Glycogen (mg/mg prot)$=1.11\times(y\times V_{Sample})\div(V_{Sample}\times Cpr)\times n$**$=1.11\times y\div Cpr\times n$**

4. Calculated by the number of bacteria or cells

Glycogen (mg/10^4)$=1.11\times(y\times V_{Sample})\div$(the number of bacteria or cells$\times V_{Sample}\div V_{Total\ sample})\times n$**$=5.55\times y\div$the number of bacteria or cells$\times n$**

Where: 1.11: 100 μg glucose with anthrone reagent color equivalent to 111 μg glycogen. V_{Sample}: the sample volume to be tested, 0.06 mL. W: sample mass, g. $V_{Total\ Sample}$: sample volume, 5 mL. n: the sample dilution factor. Cpr: Sample protein concentration, mg/mL. The number of bacteria or cells: 10^4 as the unit, ten thousand.

Typical Data

Typical standard curve:

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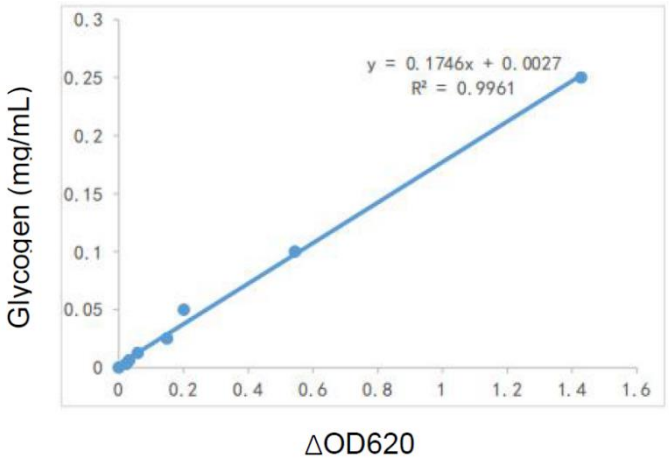

Figure 1. Standard curve of glycogen, data provided for demonstration purposes only. A new standard curve must be generated for each assay.

Disclaimer

The reagent is only used in the field of scientific research, not suitable for clinical diagnosis or other purposes.

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