Glycogen Assay Kit

(Cat/No.:BC097 Size:50T/48S)

1. Assay principle (Muscle-Liver GlycogenAssay)

Glycogen can be dehydrated to form furfural derivatives under the action of concentrated sulfuric acid, which then react with anthrone to form a blue compound. The content of glycogen is quantified by colorimetric comparison with a standard glucose solution treated by the same method. Glycogen is highly stable in concentrated alkaline solution; therefore, tissues are heated in concentrated alkali to destroy other components while preserving glycogen before color development.

2. Composition & preparation (The kit is valid for 6 months)

Reagent 1: Alkaline Solution, 40 mL × 1 vial, store at room temperature or 2～8℃.

Reagent 2: 1 mg/mL Glucose Standard Stock Solution, 1 mL × 1 vial, store at room temperature or 2～8℃. Prepare the **0.1 mg/mL Glucose Standard Working Solution** before use by mixing the 1 mg/mL glucose standard stock solution with double distilled water at a ratio of 1:9. Prepare only the required amount for immediate use, valid on the day of preparation.

Reagent 3: Chromogenic Powder, 6 vials, store at room temperature or 2～8℃. For use, add 25 mL of concentrated sulfuric acid (concentrated sulfuric acid to be prepared by the user) to each vial, stir to dissolve completely, prepare fresh before use.

Notes on Chromogenic Reagent Preparation

1.The concentrated sulfuric acid must be analytical grade with a concentration of 95%-98% and should not be left open for an extended period (concentration will decrease if stored open for too long).

2.The container and graduated cylinder used for preparing the chromogenic reagent must be absolutely dry; otherwise, Reagent 3 cannot be fully dissolved.

3.Preparation procedure: Pour the powder into a beaker first, then add a small amount of concentrated sulfuric acid (about 10 mL), stir carefully with a glass rod to dissolve completely, then add the remaining concentrated sulfuric acid, stir thoroughly, and store at room temperature in the dark.

3. Required Instruments and Reagents

Visible spectrophotometer with 1 cm light path cuvettes, boiling water bath, electronic balance, distilled water, normal saline, concentrated sulfuric acid (analytical grade), vortex mixer, beakers and glass rods.

4. Sample Pretreatment

1.Tissue Sample Treatment：

① **Sampling:** Rinse tissue samples (e.g., liver or muscle) with normal saline, blot dry with filter paper, and weigh (sample weight ≤ 100 mg is recommended, do not exceed 100 mg).

② **Hydrolysis:** Add the sample and alkaline solution into a test tube at a ratio of sample weight (mg) : alkaline solution volume (μL) = 1:3, heat in a boiling water bath for 20 min, then cool under running water.

Example: For a 75 mg liver tissue sample, the volume of alkaline solution to add at a 1:3 weight-to-volume ratio is 225 μL; for an 85 mg muscle tissue sample, the volume of alkaline solution to add at a 1:3 weight-to-volume ratio is 255 μL.

③ **Further prepare glycogen hydrolysate into glycogen assay solution:**

Liver glycogen assay solution (1% concentration): Volume of double distilled water to add = Liver weight × 100 - Liver weight × 4* = Liver weight × 96

Muscle glycogen assay solution (5% concentration): Volume of double distilled water to add = Muscle weight × 20 - Muscle weight × 4* = Muscle weight × 16

Note: 4 refers to the total volume of alkaline solution and tissue during hydrolysis.

Example as above: For preparing 1% liver glycogen assay solution, the volume of double distilled water to add = 75 × 96 = 7200 μL = 7.2 mL; for preparing 5% muscle glycogen assay solution, the volume of double distilled water to add = 85 × 16 = 1360 μL = 1.36 mL.

2. Cell Sample Pretreatment

① Collection of cell pellets (cell number ≥ 1×10^6):

(1) For suspension-cultured cells: Take the cell suspension directly, centrifuge at 1000 rpm for 10 min, discard the supernatant and retain the cell pellet.

(2) For adherent-cultured cells: Detach cells with trypsin or scrape cells with a cell scraper to prepare a cell suspension, centrifuge at 1000 rpm for 10 min, discard the supernatant and retain the cell pellet.② Washing of cell pellets:Add 0.5～1 mL of buffer (0.1 mol/L phosphate buffer pH 7～7.4 or normal saline) to the cell pellet, mix gently, centrifuge at 1000 rpm for 10 min, discard the supernatant and retain the cell pellet.

③ Hydrolysis:

(1) Hydrolysis after disruption (suitable for samples with unknown or highly variable cell numbers): Add 0.2～0.5 mL of buffer (0.1 mol/L phosphate buffer pH 7～7.4 or normal saline) to the cell pellet, disrupt cells by ultrasonic treatment on ice bath or manual homogenization (no centrifugation). Take 0.05 mL of the homogenate (a portion can be used to determine total protein concentration; total protein assay kits are available from our company, recommended A045-3/-4, BCA Total Protein Assay Kit),

add 0.15 mL of alkaline solution, heat in a boiling water bath for 20 min, cool under running water—this is the **glycogen assay solution**.

(2) Direct hydrolysis without disruption (suitable for samples with similar total cell numbers or when total cell numbers are not a consideration): Add 0.25 mL of alkaline solution to each tube, heat in a boiling water bath for 20 min, cool under running water, add 0.25 mL of double distilled water—this is the **glycogen assay solution**.

5. Operation Table

	Blank	Standard	Sample
DDW (mL)	1.0	0.9	0.9
0.1 mg/mL Standard (mL)		0.1	
glycogen assay solution (mL)			0.1
Chromogenic Solution (mL)	2	2	2
Mix well, incubate in boiling water for 5 min, take out and cool, mix well again. Measure the absorbance (A) of each tube at 620 nm with a 1 cm light path, using the blank tube to zero the spectrophotometer.			

[Note]: The chromogenic solution must be thoroughly mixed before heating in the boiling water bath; otherwise, flocculent precipitates will form.

6. Calculation Formula

1.Tissue Sample Calculation Formula：

Glycogen content(mg/g tissue)=$\frac{A_{sample}}{A_{standard}} \times C_{standard} \div C_{hydrolysate} \div 1.11$

$C_{standard}$：Concentration of standard solution, 0.1 mg/mL;

$C_{hydrolysate}$:Concentration of sample hydrolysate (0.01 g/mL for 1% liver glycogen solution, 0.05 g/mL for 5% muscle glycogen solution); (Note: Glycogen content in liver and muscle varies among species. For the first experiment, prepare all hydrolysates at a 5% concentration initially, then perform a pre-experiment to check the concentration. If the concentration is too high, dilute the solution before assay and multiply by the dilution factor in the calculation.)

1.11: Conversion factor for glucose content to glycogen content in this method, meaning the color developed by 100 μg of glycogen with anthrone reagent is equivalent to that developed by 111 μg of glucose with anthrone reagent.

2.Cell Sample Calculation Formula:

① For cells hydrolyzed after disruption:

Glycogen content(mg/g gprot)=$\frac{A_{sample}}{A_{standard}} \times C_{standard} \div 1.11 \div Cpr$

② For cells hydrolyzed directly without disruption:

Glycogen total(mg)=$\frac{A_{sample}}{A_{standard}} \times C_{standard} \div 1.11 \times V_{sample\ total}$

$C_{standard}$:Concentration of standard solution, 0.1 mg/mL;

Cpr :Protein concentration of cell homogenate, mgprot/mL (prot = protein);

$V_{sample\ total}$:Total volume of prepared glycogen assay solution, 0.5 mL;

1.11: Conversion factor for glucose content to glycogen content in this method, meaning the color developed by 100 μg of glycogen with anthrone reagent is equivalent to that developed by 111 μg of glucose with anthrone reagent.

7. Calculation Examples

Example 1: Take the liver of mice and follow the above steps. The OD value of the standard tube is measured to be 0.174, and the OD value of the test tube is 0.223.

The calculation result is:

Mouse liver glycogen content(mg/g liver tissue)= $\frac{0.233}{0.174} \times 0.1 \div 0.01 \div 1.11 = 11.546\text{mg/g liver tissue}$

Example 2: The muscle of mice was taken and processed according to the above steps. The OD value of the standard tube was measured to be 0.174, and the OD value of the test tube was 0.171.

The calculation result was:

Mouse muscle glycogen content(mg/g muscle tissue)= $\frac{0.171}{0.174} \times 0.1 \div 0.05 \div 1.11 = 1.771\ \text{mg/g muscle tissue}$

Appendix Ⅰ: Glycogen Assay for Fatty Liver Samples

一. Sample Pretreatment

1.Sampling: Rinse fresh liver samples with normal saline, blot dry with filter paper, and weigh (sample weight ≤ 100 mg is recommended, do not exceed 100 mg).

2.Hydrolysis: Add the sample and alkaline solution into a test tube at a ratio of sample weight (mg) : alkaline solution volume (μL) = 1:3, heat in a boiling water bath for 20 min, then cool under running water.

3.Further prepare glycogen hydrolysate into glycogen assay solution:Liver glycogen assay solution (5% concentration): Volume of double distilled water to add = Liver weight × 20 - Liver weight × 4 = Liver weight × 16 (**Note: 4 refers to the total volume of alkaline solution and tissue during hydrolysis**).

4.Defatting of assay solution: Take the prepared assay solution (a milky white oily substance is usually floating on the upper layer), add a certain volume of chloroform at a ratio of assay solution (mL) : chloroform (mL) = 4:1, mix on a vortex mixer, centrifuge at 3500 rpm for 10 min, take the supernatant for assay (dilute the supernatant if the glycogen content is too high).

二. Operation Table

	Blank	Standard	Sample
DDW (mL)	1.0	0.9	0.9
0.1mg/mL Standard (mL)		0.1	
glycogen assay solution (mL)			0.1
Chromogenic Solution (mL)	2	2	2

Mix well, heat in boiling water for 5 min, take out and cool, mix well again. Measure the absorbance (A) of each tube at 620 nm with a 1 cm light path, using the blank tube to zero the spectrophotometer.

三. Calculation and Example

1. Calculation formula

Glycogen content(mg/g tissue)= $\frac{A_{sample}}{A_{standard}} \times C_{standard} \div C_{hydrolysate} \div 1.11$

$C_{standard}$**:**Concentration of standard solution, 0.1 mg/mL;

$C_{hydrolysate}$**:**Concentration of sample hydrolysate (0.05 g/mL for 5% liver glycogen solution);

1.11: Conversion factor for glucose content to glycogen content in this method, meaning the color developed by 100 μg of glycogen with anthrone reagent is equivalent to that developed by 111 μg of glucose with anthrone reagent.

2.Calculation Example

Liver samples from fatty liver model rats were processed following the procedures in the above appendix. The absorbance (OD) value of the standard tube was measured as 0.174, and that of the assay tube as 0.348. The calculated result is as follows:

Mouse liver glycogen content(mg/g liver tissue)= $\frac{0.348}{0.174} \times 0.1 \div 0.05 \div 1.11 = 3.603$ mg g liver tissue