Technical Manual

Glycogen Fluorometric Assay Kit

- **Catalogue Code: MAES0011**
- **Size: 96T**
- **Research Use Only**

1. Key features and Sample Types

Detection method:

Fluorimetric method

Specification:

96T

Range:

0.06-4.0 µg/mL

Sensitivity:

0.06 µg/mL

Storage:

-20°C for 6 months

Expiry:

See Kit Label

Experiment Notes:

This kit is for **research use only.**

Instructions should be strictly followed. Changes of operation may result in unreliable results.

The validity of kit is 6 months.

Do not use components from different batches of kit.

2. Background

Glycogen is mainly produced by liver and muscle. Glycogen content is mainly regulated by glycogen synthase and glycogen phosphorylase. In the liver, glycogen acts as a glucose store for other tissues and maintains blood glucose levels. In muscle, glycogen is mainly used as energy for the supply of adenosine triphosphate (ATP) during muscle contraction. Due to the lack of glucose-6-phosphatase, muscle glycogen cannot maintain the level blood glucose.

3. Intended Use

This kit can be used for determination of glycogen content in animal liver and muscle tissue samples.

4. Detection Principle

Glycogen produces glucose under the action of starch glycosidase, and glucose is catalyzed by glucose oxidase to produce hydrogen peroxide. In the presence of the peroxidase, hydrogen peroxide be oxidized to produce the fluorescence substrate. The fluorescence intensity at the excitation wavelength of 535 nm and emission wavelength of 587 nm is proportional to the glycogen content.

5. Kit components & storage

Item	Specification	Storage
Buffer Solution A	30 mL × 1 vial	-20°C, 6 months
Buffer Solution B	8 mL × 1 vial	-20°C, 6 months
Probe	0.24 mL × 1 vial	-20°C, 6 months, avoid direct sunlight
Enzyme Reagent A	Lyophilized × 1 vial	-20°C, 6 months
Enzyme Reagent B	Lyophilized ×1 vial	-20°C, 6 months
Glucogen Standard Solution (0.1 mg/mL)	0.5 mL × 1 vial	-20°C, 6 months
Black Microplate	96 wells	No requirement
Plate Sealer	2 pieces	

Materials required but not supplied

- Micropipettor
- Incubator
- Centrifuge
- Fluorescence microplate reader (Ex/Em=535 nm/590 nm)
- Tips (10 µL, 200 µL, 1000 µL)
- EP tubes (1.5 mL, 2 mL)
- Double distilled water

6. Assay Notes:

1. After preparation of the reaction working solution, it must be stored with avoid direct sunlight.

2. Prevent the formulation of bubbles when the reagents is added into the microplate.

3. Since the tissues continue to have a relatively high rate of anaerobic metabolism after death, the glucose content in the tissues decreases rapidly to undetectable levels, resulting in further hydrolysis of glycogen and a significant decrease of the content. To accurately measure tissue glycogen, if it is not possible to measure it immediately, effective inactivation means should be adopted after the sample is taken out. The tissue can be immediately moved to liquid nitrogen, and then ground in liquid nitrogen and stored at -20 or -80°C.

7. Reagent Preparation

1. Bring all reagent to room temperature before use.

2. Preparation of **enzyme working solution A:** Dissolve enzyme reagent A with 1.2 mL of buffer solution A. Prepare the needed amount before use. The prepared solution can be aliquoted and stored at -20°C for 1 week.

3. Preparation of **enzyme working solution B:** Dissolve enzyme reagent B with 0.24 mL of buffer solution B. Prepare the needed amount before use. The prepared solution can be aliquoted and stored at -20°C for 1 week.

4. Preparation of **reaction working solution:** Mix the buffer solution B, probe and enzyme working solution B at a ratio of 46:2:2 fully. Prepare the fresh solution before use and store it with avoid direct sunlight.

5. Preparation of **glucogen standard (25 µg/mL):** Dilute 100 µL of 0.1 mg/mL glucogen standard solution with 300 µL of buffer solution A and mix fully.

8. Sample Preparation

1. Cell sample:
Add double distilled water at a ratio of cell number (106): homogenization medium (µL) = 1: 200. Sonicate or mechanical homogenate and incubate at 95°C for 10 min. Cool with ice water and centrifuge at 12000 g at 4°C for 10 min, then take the supernatant and preserve it on ice for detection.

2. Tissue sample:
Take 0.1g fresh tissue, add 0.9 mL of double distilled water according to the ratio of Weight (g): Volume (mL) =1:9. Mechanical homogenate the sample in ice water bath and incubate at 95°C for 10 min. Cool with ice water and centrifuge at 12000 g for 10 min at 4°C, then take the supernatant for detection.

Sample Notes:

The concentration should be determined before preforming the assay. If the sample concentration is not within the range of the standard curve, users must determine the optimal sample dilutions for their particular experiments.

If the sample type is not included in the manual, a preliminary experiment is suggested to verify the validity.

Dilution of Samples:

Large variances in results may be seen when performing pre-experiments. Dilute the sample according to the result of the pre-experiment and the detection range (0.06-4.0 μg/mL).

The recommended dilution factor for different samples is as follows (for reference only).

Sample Type:	Dilution Factor
10% Rat liver tissue homogenate	3000-5000
10% Mouse muscle tissue homogenate	10-20

Note: The diluent is buffer solution A. Liver samples can be diluted step by step.

9. Assay Protocol

Ambient Temperature: 25-30°C

Optimum detection wavelength: Ex/Em=535 nm/587 nm

Plate Set Up:

	1	2	3	4	5	6	7	8	9	10	11	12
A	A	A	S1	S1'	S9	S9'	S17	S17'	S25	S25'	S33	S33'
B	B	B	S2	S2'	S10	S10'	S18	S18'	S26	S26'	S34	S34'
C	C	C	S3	S3'	S11	S11'	S19	S19'	S27	S27'	S35	S35'
D	D	D	S4	S4'	S12	S12'	S20	S20'	S28	S28'	S36	S36'
E	E	E	S5	S5'	S13	S13'	S21	S21'	S29	S29'	S37	S37'
F	F	F	S6	S6'	S14	S14'	S22	S22'	S30	S30'	S38	S38'
G	G	G	S7	S7'	S15	S15'	S23	S23'	S31	S31'	S39	S39'
H	H	H	S8	S8'	S16	S16'	S24	S24'	S32	S32'	S40	S40'

Note: A-H, standard wells; S1-S40, sample wells; S1'- S40', control wells.

10. Operation Steps

The preparation of standard curve

Dilute standard (25 μg/mL) with buffer solution A to a serial concentration. The recommended dilution gradient is as follows: 0, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 4.0 μg/mL.

The measurement of samples

1. **Standard well:** Add 50 μL of standard with different concentrations into the well.
   Sample well: Add 50 μL of sample into the wells.
   Conrtol well: Add 50 μL of sample into the wells.

2. Add 20 μL of enzyme working solution A to standard well and sample well.

3. Add 20 μL of buffer solution A to control well.

4. Add 50 μL of reaction working solution to each well.

5. Mix fully with microplate reader for 5 s and stand at room temperature for 30 min with avoid direct sunlight. Measure the fluorescence intensity at the excitation wavelength of 535 nm and the emission wavelength of 587 nm. The fluorescence intensity of sample well recorded as F_1, and the fluorescence intensity of control well recorded as F_2.

Operation Table

	Standard well	Sample well	Control well
Standard with different concentrations (μL)	50		
Sample (μL)		50	50
Enzyme working solution A (μL)	20	20	
Buffer solution A (μL)			20
Reaction working solution (μL)	50	50	50
Mix fully with microplate reader for 5 s and stand at room temperature for 30 min with avoid direct sunlight. Measure the fluorescence intensity at the excitation wavelength of 535 nm and the emission wavelength of 587 nm. The fluorescence intensity of sample well recorded as F_1, and the fluorescence intensity of control well recorded as F_2.			

11. Calculations

Plot the standard curve by using fluorescence value (F) of standard and correspondent concentration as y-axis and x-axis respectively. Create the standard curve with graph software (or EXCEL). The concentration of the sample can be calculated according to the formula based on the F value of sample. The standard curve is: y= ax + b.

1. Cell sample:

Glycogen content (μg/10^6 cells)

$= (\Delta F- b) \div a \times f \div (n \div V_1)$

2. Tissue sample:

Glycogen content (μg/mg wet weight)

$= (\Delta F- b) \div a \times f \div (m \div V_2)$

y: The absolute fluorescence value of standard, $F_{Standard} - F_{Blank}$ (F_{Blank} is the F value when the standard concentration is 0)

x: The concentration of standard

a: The slope of standard curve

b: The intercept of standard curve

ΔF: Change of fluorescence intensity of sample $(F_1 - F_2) - F_{Blank}$.

f: Dilution factor of sample before tested

m: The weight of tissue sample, 100 mg

n: The number of cells. For example, the number of cells is $5*10^6$, n is 5

V_1: The volume of double distilled water added during the preparation of cells sample, mL

V_2: The volume of double distilled water added during the preparation of tissue sample, mL

12. Performance Characteristics

Detection Range	0.06-4.0 μg/mL
Sensitivity	0.06 μg/mL
Average inter-assay CV (%)	6.6
Average intra-assay CV (%)	3.4

Analysis

For mouse liver tissue, take 50 μL of tissue supernatant diluted for 4000 times, carry the assay according to the operation table.

The results are as follows:

standard curve: y = 2283 x + 106.49, the average fluorescence value of the sample (F1) is 3961, the average fluorescence value of the control (F2) is 1103, the average OD value of the blank is1444, △F = 3961 – 1103 – 1444 = 1414, and the calculation result is:

Glycogen content (μg/mg wet weight)

= (1414-106.49) ÷ 2283 × 4000 ÷ (100÷0.9)
= 20.62 μg/mg wet weight

Safety Notes

Some of the reagents in the kit contain dangerous substances. Prevent touching skin and clothing.

Wash immediately with plenty of water if touching it carelessly.

All the samples and waste material should be treated according to the relevant rules of laboratory's biosafety.

Before the experiment, read the instructions carefully, and wear gloves and work clothes.

Email: info@assaygenie.com **Web:** www.assaygenie.com

Notes:

Email: info@assaygenie.com **Web:** www.assaygenie.com

Notes:

Email: info@assaygenie.com **Web:** www.assaygenie.com

Notes:

Email: info@assaygenie.com **Web:** www.assaygenie.com

Assay Genie 100% money-back guarantee!

If you are not satisfied with the quality of our products and our technical team cannot resolve your problem, we will give you 100% of your money back.

Contact Details

Email: info@assaygenie.com

Web: www.assayenie.com