（FOR RESEARCH USE ONLY. DO NOT USE IT IN CLINICAL DIAGNOSIS !）

Catalog No：E-BC-F040

Specification：96T(40 samples)

Measuring instrument：Fluorescence Microplate Reader

(Ex/Em=535 nm/587 nm)

Detection range：0.06-4.0 μg/mL

Elabscience® Glycogen Fluorometric Assay Kit

This manual must be read attentively and completely before using this product.

If you have any problem, please contact our Technical Service Center for help：

Toll-free: 1-888-852-8623

Tel: 1-832-243-6086

Fax: 1-832-243-6017

Email: techsupport@elabscience.com

Website: www.elabscience.com

Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

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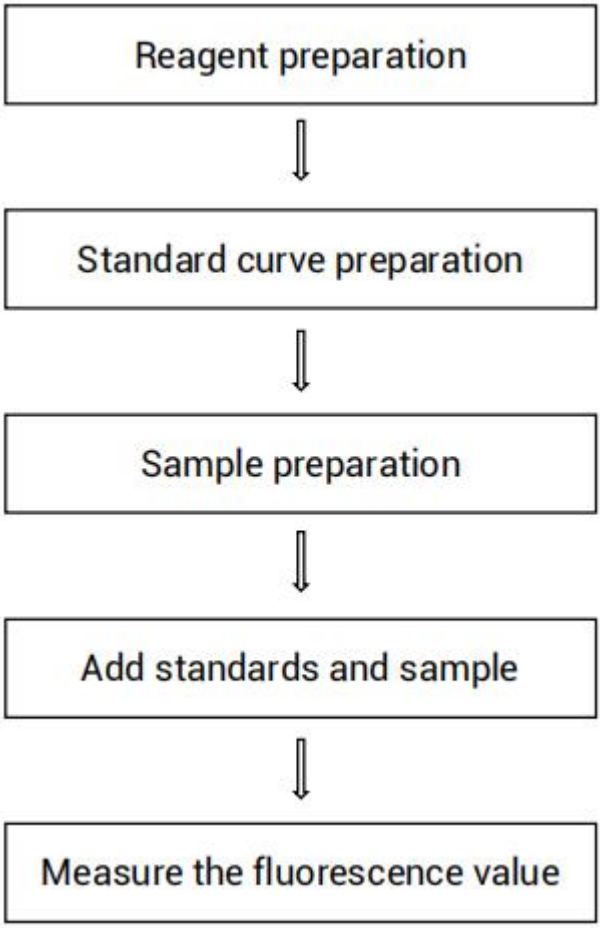
Assay summary



Intended use

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

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.

Kit components & storage

Item	Component	Size (96 T)	Storage
Reagent 1	Buffer Solution A	30 mL × 1 vial	-20℃, 12 months
Reagent 2	Buffer Solution B	8 mL × 1 vial	-20℃, 12 months
Reagent 3	Probe	0.24 mL × 1 vial	-20℃, 12 months shading light
Reagent 4	Enzyme Reagent A	Powder × 1 vial	-20℃, 12 months
Reagent 5	Enzyme Reagent B	Powder × 1 vial	-20℃, 12 months
Reagent 6	0.1 mg/mL Glucogen Standard Solution	0.5 mL × 1 vial	-20℃, 12 months
	Black Microplate	96 wells	No requirement
	Plate Sealer	2 pieces	
	Sample Layout Sheet	1 piece	

Note: The reagents must be stored strictly according to the preservation conditions in the above table. The reagents in different kits cannot be mixed with each other. For a small volume of reagents, please centrifuge before use, so as not to obtain sufficient amount of reagents.

Materials prepared by users

Instruments:

Micropipettor, Incubator, Centrifuge, Fluorescence microplate reader (Ex/Em=535 nm/587 nm)

Reagent preparation

① Equilibrate all the reagents to room temperature before use.

② The preparation of enzyme working solution A:
Dissolve one vial of enzyme reagent A with 1.2 mL of buffer solution A, mix well to dissolve. Aliquoted storage at -20°C for 7 days.

③ The preparation of enzyme working solution B:
Dissolve one vial of enzyme reagent B with 240 μL of buffer solution B, mix well to dissolve. Aliquoted storage at 2-8°C for 7 days.

④ The preparation of reaction working solution:
For each well, prepare 50 μL of measuring working solution (mix well 46 μL of buffer solution B, 2 μL of probe and 2 μL of enzyme working solution B). The enzyme working solution should be prepared on spot and protected from light.

⑤ The preparation of 25 μg/mL glucogen standard :
Dilute 100 μL of 0.1 mg/mL glucogen standard solution with 300 μL of buffer solution A and mix fully.

⑥ The preparation of standard curve：
Always prepare a fresh set of standards. Discard working standard dilutions after use.
Dilute 25 μg/mL glucogen standard 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. Reference is as follows:

Item	①	②	③	④	⑤	⑥	⑦	⑧
Concentration (μg/mL)	**0**	**0.5**	**1.0**	**1.5**	**2.0**	**2.5**	**3.0**	**4.0**
25 μg/mL standard (μL)	0	10	20	30	40	50	60	80
Buffer solution A (μL)	500	490	480	470	460	450	440	420

Sample preparation

① Sample preparation

Tissue sample:

① Harvest the amount of tissue needed for each assay (initial recommendation 20 mg).

② Wash tissue in cold PBS (0.01 M, pH 7.4).

③ Homogenize 20 mg tissue in 180 μL double distilled water with a dounce homogenizer at 4℃.

④ Mechanical homogenate the sample in ice water bath and incubate at 95℃ for 10 min.

⑤ Centrifuge at 10000×g for 10 min to remove insoluble material. Collect supernatant and keep it on ice for detection.

Cell (adherent or suspension) samples:

① Harvest the number of cells needed for each assay (initial recommendation 1×10^6 cells).

② Wash cells with PBS (0.01 M, pH 7.4).

③ Add 1×10^6 cells in 200 μL double distilled water to 2 mL EP tube. Sonicate or mechanical homogenate and incubate at 95℃ for 10 min

④ Centrifuge at 12000×g for 10 minutes to remove insoluble material. Collect supernatant and keep it on ice for detection.

② **Dilution of sample**

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. For the dilution of other sample types, please do pretest to confirm the dilution factor.

The key points of the assay

① After preparation of the reaction working solution, it must be stored with shading light..

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℃.

Operating steps

① Standard well: add 50 μL of standard with different concentrations into the well.

Sample well: add 50 μL of sample into the well.

Control well: add 50 μL of sample into the well.

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

Add 20 μL of buffer solution A to control well.

③ Add 50 μL of reaction working solution to each well.

④ Mix fully with microplate reader for 5 s and stand at room temperature for 30 min with shading light. 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.

Calculation

The standard curve:

1. Average the duplicate reading for each standard.

2. Subtract the mean fluorescence value of the blank (Standard #①) from all standard readings. This is the absoluted fluorescence value.

3. Plot the standard curve by using absoluted fluorescence value of standard and correspondent concentration as y-axis and x-axis respectively. Create the standard curve (**y = ax + b**) with graph software (or EXCEL).

The sample:

1. Cell sample:

$$\text{Glycogen content (μg/10^6 cells)} = (\Delta F - b) \div a \times f \div (n \div V_1)$$

2. Tissue sample:

$$\text{Glycogen content (μg/mg wet weight)} = (\Delta F - b) \div a \times f \div (m \div V_2)$$

[Note]

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.

Appendix I Performance Characteristics

1. Parameter:

Intra-assay Precision

Three rat liver samples were assayed in replicates of 20 to determine precision within an assay. (CV = Coefficient of Variation)

Parameters	Sample 1	Sample 2	Sample 3
Mean (μg/mL)	0.15	1.00	2.50
%CV	4.0	3.2	3.0

Inter-assay Precision

Three rat liver samples were assayed 20 times in duplicate by three operators to determine precision between assays.

Parameters	Sample 1	Sample 2	Sample 3
Mean (μg/mL)	0.15	1.00	2.50
%CV	7.3	6.2	6.3

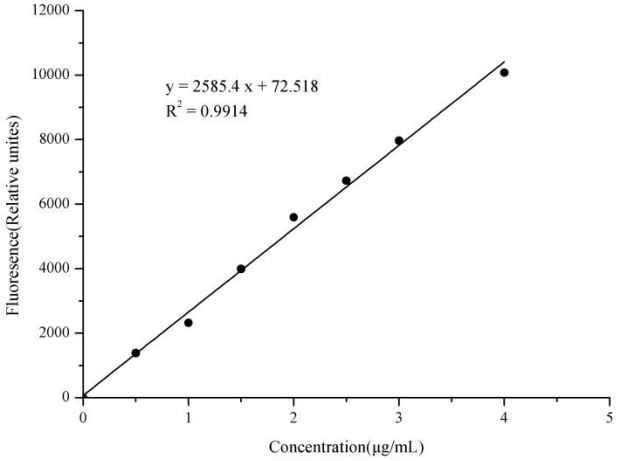
Sensitivity

The analytical sensitivity of the assay is 0.06 μmol/L. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 20 times, and calculating the corresponding concentration.

2. Standard curve:

As the fluorescence value of the standard curve may vary according to the conditions of the actual assay performance (e.g. operator, pipetting technique or temperature effects), so the standard curve and data are provided as below for reference only：

Concentration (μg/mL)	**0**	**0.5**	**1.0**	**1.5**	**2.0**	**2.5**	**3.0**	**4.0**
Fluorescence value	1269	2695	3650	5304	6826	8119	9197	11360
	1257	2599	3519	5209	6885	7861	9271	11325
Average fluorescence value	1263	2647	3585	5257	6856	7990	9234	11343
Absoluted fluorescence value	0	1384	2322	3994	5593	6727	7971	10080



Appendix Π Example Analysis

Example analysis：

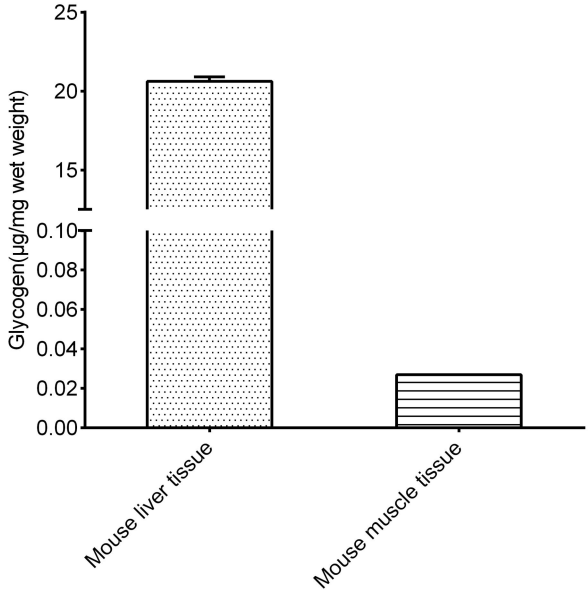
For mouse liver tissue, take 50 μL of tissue supernatant diluted for 4000 times and carry the assay according to the operation steps. The results are as follows:

standard curve: y = 2283 x + 106.49, the average fluorescence value of the sample (F_1) is 3961, the average fluorescence value of the control (F_2) 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

Detect 10% mouse liver tissue homogenate (dilute for 4000 times) and 10% mouse muscle tissue homogenate (dilute for 10 times) according to the protocol, the result is as follows：



Statement

1. This assay kit is for Research Use Only. We will not response for any arising problems or legal responsibilities causing by using the kit for clinical diagnosis or other purpose.
2. Please read the instructions carefully and adjust the instruments before the experiments. Please follow the instructions strictly during the experiments.
3. Protection methods must be taken by wearing lab coat and latex gloves.
4. If the concentration of substance is not within the detection range exactly, an extra dilution or concentration should be taken for the sample.
5. It is recommended to take a pre-test if your sample is not listed in the instruction book.
6. The experimental results are closely related to the situation of reagents, operations, environment and so on. Elabscience will guarantee the quality of the kits only, and NOT be responsible for the sample consumption caused by using the assay kits. It is better to calculate the possible usage of sample and reserve sufficient samples before use.