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

Catalog No：E-BC-F171

Specification：96T(80 samples)

Measuring instrument：Fluorescence Microplate Reader

(Ex/Em=535 nm/587 nm)

Detection range：0.014-4 μg/mL

Elabscience® Glycogen Fluorometric Assay Kit

(Glycosidase Method)

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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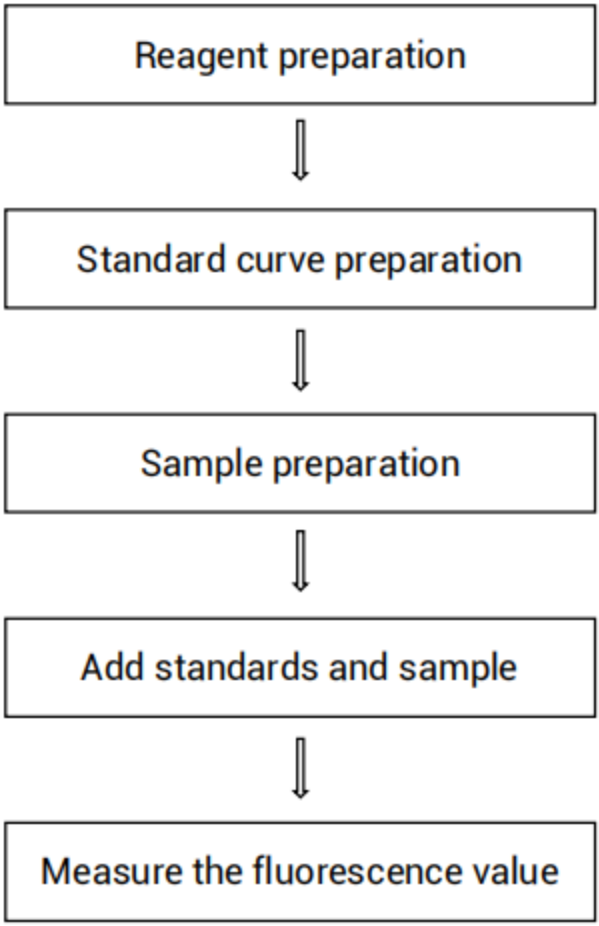
Assay summary



Intended use

This kit can be used to measure glycogen content in animal liver, muscle tissue and hepatocyte, myocyte samples.

Detection principle

Glycogen is converted into glucose under the action of starch glycosidase. Glucose is then catalyzed by glucose oxidase to produce hydrogen peroxide. Under the action of peroxidase, the non-fluorescent probe is oxidized to a fluorescent substance, and its fluorescence intensity is related to the content of glycogen.

Kit components & storage

Item	Component	Size (96 T)	Storage
Reagent 1	Buffer Solution A	55 mL × 1 vial	-20°C, 12 months
Reagent 2	Buffer Solution B	8 mL × 1 vial	-20°C, 12 months
Reagent 3	Probe	0.24 mL × 1 vial	-20°C, 12 months shading light
Reagent 4	Enzyme Reagent A	Powder × 2 vials	-20°C, 12 months
Reagent 5	Enzyme Reagent B	Powder × 1 vial	-20°C, 12 months
Reagent 6	0.1 mg/mL Glucogen Standard Solution	0.5 mL × 1 vial	-20°C, 12 months
Reagent 7	Chromogenic Agent	2.4 mL × 1 vial	-20°C, 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:

Fluorescence microplate reader (Ex/Em=535 nm/587 nm), Incubator, Water bath.

Reagent preparation

① Keep enzyme reagent A and enzyme reagent B on ice , equilibrate other reagents to 25°C before use.

② The preparation of enzyme reagent A working solution :
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 reagent B working solution :
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 A:
Before testing, please prepare sufficient reaction working solution A according to the test wells. For example, prepare 500 μL of reaction working solution A (mix well 280 μL of buffer solution B, 20 μL of enzyme reagent B working solution and 200 μL of chromogenic agent). The reaction working solution A should be prepared on spot protected from light and used up within 8 h.

⑤ The preparation of reaction working solution B:

Before testing, please prepare sufficient reaction working solution B according to the test wells. For example, prepare 500 μL of reaction working solution B (mix well 280 μL of buffer solution B, 20 μL of probe and 200 μL of enzyme reagent A working solution). The reaction working solution B should be prepared on spot protected from light and used up within 8 h.

⑥ 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. The prepared solution should be used up within 8 h.

⑦ 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 Homogenize 20 mg tissue in 180 μL double distilled water with a dounce homogenizer at 4°C.

③ Subject the homogenate to a boiling water bath for 10 min immediately and cool down to 25°C with running water.

④ Centrifuge at 12000×g for 10 min at 4°C to remove insoluble material. Collect supernatant and keep it on ice for detection. The supernatant should be used up within 8 h.

Cell samples:

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

② Homogenize 1×10^6 cells in 200 μL of double distilled water with a ultrasonic cell disruptor at 4°C.

③ Subject the homogenate to a boiling water bath for 10 min immediately and cool down to 25°C with running water.

④ Centrifuge at 12000×g for 10 min to remove insoluble material. Collect supernatant and keep it on ice for detection. The supernatant should be used up within 8 h.

② **Dilution of sample**

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

Sample type	Dilution factor
10% Mouse liver tissue homogenate	200-400
10% Mouse muscle tissue homogenate	5-10
1×10^6 HepG2 cells	1

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

Due to the rapid anaerobic metabolic rate that persists in tissues after an animal's 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. If samples cannot be tested immediately after collection, effective inactivation measures must be applied upon extraction. The tissue can be immediately moved to liquid nitrogen, and then ground in liquid nitrogen and stored at -20°C or -80°C.

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.

② Add 50 μL of reaction working solution A to each well.

③ Mix fully with fluorescence microplate for 5s and Incubate at 37°C for 30 min.

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

⑤ Mix fully and determine the fluorescence value under the conditions of an excitation wavelength of 535 nm and an emission wavelength of 587 nm, as F_1.

⑥ Incubate at 37°C for 30 min, determine the fluorescence value under the conditions of an excitation wavelength of 535 nm and an emission wavelength of 587 nm, as F_2, $\Delta F = F_2 - F_1$.

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. Tissue sample:

$$\text{Glycogen content (μg/mg wet weight)} = \frac{\Delta F_{sample} - \Delta F_{blank} - b}{a} \div m \times V_1 \times f$$

2. Cell sample:

$$\text{Glycogen content (μg/10^6)} = \frac{\Delta F_{sample} - \Delta F_{blank} - b}{a} \div n \times V_2 \times f$$

[Note]

m: The weight of sample, mg.

V_1: The volume of double distilled water added during tissue homogenate, 0.9 mL.

n: The number of cells/10^6.

V_2: The volume of double distilled water added during cell homogenate, 0.2 mL.

f: Dilution factor of sample before tested.

Appendix I Performance Characteristics

1. Parameter:

Intra-assay Precision

Three 10% mouse liver tissue homogenate 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)	1.25	2.25	3.25
%CV	4.1	5.2	3.7

Inter-assay Precision

Three 10% mouse liver tissue homogenate 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)	1.5	2.5	3.5
%CV	3.2	5.9	6.0

Recovery

Take three samples of high concentration, middle concentration and low concentration to test the samples of each concentration for 6 times parallelly to get the average recovery rate of 96.3%.

	Sample 1	Sample 2	Sample 3
Expected Conc. (μg/mL)	1.0	2.0	3.0
Observed Conc. (μg/mL)	0.99	1.93	2.83
Recovery rate (%)	99	96	94

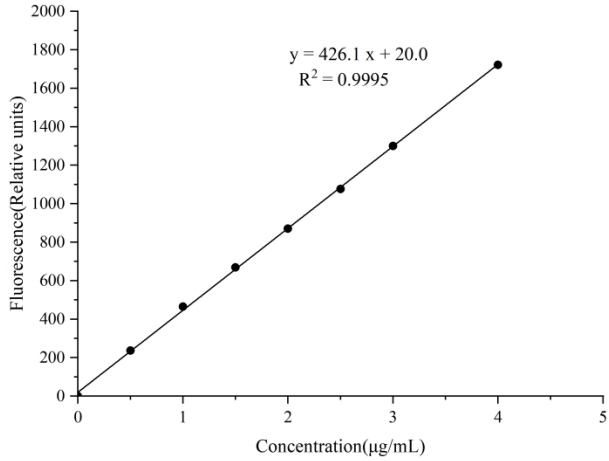
Sensitivity

The analytical sensitivity of the assay is 0.014 μg/mL. 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
F_1	294	314	337	339	356	358	360	381
	290	304	345	333	342	351	359	377
F_2	609	869	1115	1349	1529	1770	1997	2426
	610	858	1133	1295	1544	1727	1956	2409
ΔF	315	555	778	1010	1173	1412	1637	2045
	320	554	788	962	1202	1376	1597	2032
Average fluorescence value	318	555	783	986	1188	1394	1617	2039
Absoluted fluorescence value	0	237	466	669	870	1077	1300	1721



Appendix Π Example Analysis

Example analysis:

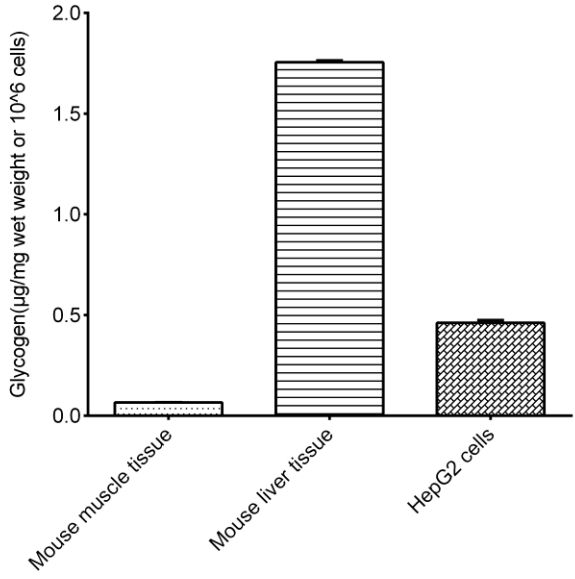
Take 50 μL of 10% mouse liver tissue homogenate, diluted for 400 times and carry the assay according to the operation steps. The results are as follows:

standard curve: y = 426.1 x + 20.0, the F_1 value of the blank well is 292, the F_2 value of the blank well is 610, ΔF_{blank} = 610 - 292 = 318, the F_1 value of the sample well is 300, the F_2 value of the sample well is 845, ΔF_{sample}= 845 - 300 = 545, and the calculation result is:

Glycogen content (μg/mg wet weight) = (545 - 318 - 20.0) ÷ 426.1 ÷ 100 × 0.9 × 400

= 1.75 μg/mg wet weight

Detect 10% mouse muscle tissue homogenate (dilute for 8 times) , 10% mouse liver tissue homogenate (dilute for 400 times) and 1×10^6 HepG2 cells 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.