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

Catalog No：E-BC-K073-S

Specification：50 assays(48 samples)/ 100 assays(96 samples)

Measuring instrument：Spectrophotometer（620 nm）

Detection range：1.80-180 mg/g liver tissue, 0.36-36 mg/g muscle tissue

Elabscience®Glycogen Colorimetric Assay Kit

(Liver/Muscle Samples)

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

Tell: 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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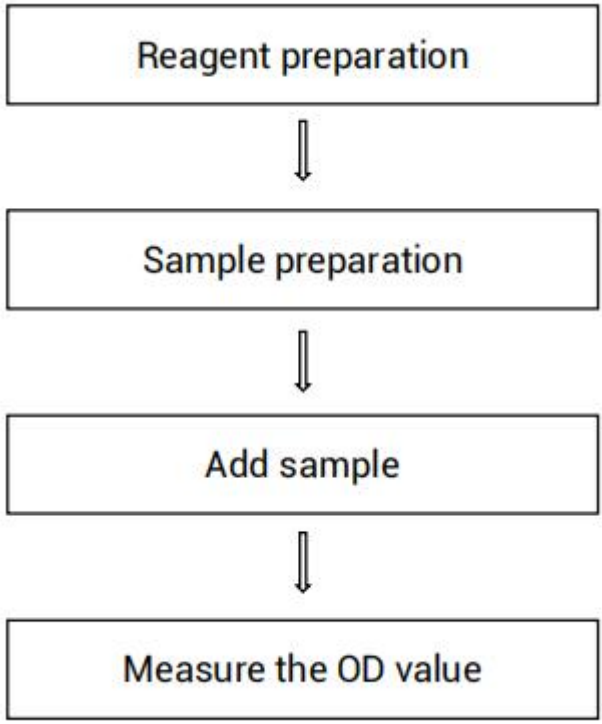
Assay summary



Intended use

This kit can be used to measure the glycogen content in animal liver and muscle samples.

Detection principle

Under the presence of concentrated sulfuric acid, glycogen can be dehydrated to furfural derivatives. Furfural derivatives can form blue compound with anthracenone. The concentration of the compound can be measured by colorimetric quantification at 620 nm with glucose standard buffer of same treatment. Glycogen is quite stable in concentrated alkali solution. Heating the tissue sample in concentrated alkali solution before color development will remove other components and keep the glycogen.

Kit components & storage

Item	Component	Size 1 (50 assays)	Size 2 (100 assays)	Storage
Reagent 1	Alkali Reagent	50 mL × 1 vial	50 mL × 2 vials	2-8℃, 12 months
Reagent 2	Glucose Standard Solution	1 mL × 1 vial	1 mL × 1 vial	2-8℃, 12 months
Reagent 3	Anthracenone	Powder × 6 vials	Powder × 12 vials	2-8℃, 12 months shading light

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:

Spectrophotometer (620 nm), Micropipettor, Incubator, Vortex mixer, Centrifuge

Reagents:

Double distilled water, Normal saline (0.9% NaCl), Concentrated sulfuric acid

Reagent preparation

① Equilibrate all reagents to room temperature before use.

② The preparation of 0.01 mg/mL glucose standard solution：
For each well，prepare 1000 μL of 0.01 mg/mL glucose standard solution (mix well 10 μL of glucose standard solution and 990 μL of double distilled water). The 0.01 mg/mL glucose standard solution should be prepared on spot. Store at -20°C for 1 day.

③ The preparation of anthracenone working solution：
Dissolve one vial of anthracenone with 20 mL of concentrated sulfuric acid (self-prepared), mix well to dissolve. Store at 2-8°C for 2 hours protected from light. If the reagents appear darkened color, it should be abandon.

[Notes]:

① The concentrated sulfuric acid must be 95%-98% analytical reagent opened recently (the concentration will decrease if it has been opened for a long time).

② The container and graduated cylinder must be absolute dry. Otherwise anthracenone cannot be dissolved thoroughly.

③ Add reagent 3 powder into the beaker, then add about 10 mL concentrated sulfuric acid. Press with a glass rod to pulverize the

powder and improve dissolving. Add the remaining concentrated sulfuric acid and mix thoroughly.

④ Pay attention to personal safety protection.

Sample preparation

① **Sample preparation**

① **Sampling**: Wash the fresh liver or muscle tissue sample with saline and dry with filter paper. Weigh the sample. It is recommended that the weight of the sample should be less than 100 mg.

② **Hydrolysis**: Homogenize 0.1 g tissue in 0.3 mL alkali reagent. Heat the tube in boiling water bath for 20 min. Cool the tube with running water.

*Notes: Seal the tube with preservative film to avoid water evaporation. Make a small hole on the film to allow vapour expanding and contracting.

③ **Prepare the hydrolyzed glycogen testing solution for measurement：**

Concentration of the liver glycogen testing solution is 1%, the volume of the double distilled water added should be: liver weight × 100 - liver weight×4* = liver weight × 96.

Concentration of the muscle glycogen testing solution is 5%, the volume of the double distilled water added should be: muscle weight × 20 - muscle weight × 4*= muscle weight × 16.

Notes: 4* is the volume of the dehydrated sample and alkali reagent mixture.

For example: weigh 80 mg liver tissue, add 240 μL alkali reagent to hydrolyze, it should add 7680 μL double distilled water to prepare 1% liver glycogen testing solution.

The key points of the assay

① This experiment must be done in glass tubes.

② The temperature of the water bath should be above 95°C.

Operating steps

① Blank tube: Take 1 mL of double distilled water into a 10 mL glass tube
Standard tube: Take 1 mL of 0.01 mg/mL glucose standard solution into a 10 mL glass tube.
Sample tube: Take 0.1 mL of liver/muscle hydrolyzed glycogen testing solution in sample preparation step into a 10 mL glass tube and add 0.9 mL of double distilled water.

② Add 2.0 mL of anthracenone working solution and oscillate fully with vortex mixer (The anthracenone should be added slowly after addition of standard or sample).

③ Fasten the tubes with plastic film and make a small hole, and incubate the tubes in 100℃ water bath for 5 min, then cool the tubes with running water immediately.
Notes: the mixture must be mix fully after adding the anthracenone working solution, then heat the tubes in boiling water bath. Otherwise floccule will be formed during heating

④ Set the spectrophotometer to zero with double distilled water and measure the OD value of each tube at 620 nm with 1 cm optical path cuvette.

Calculation

The sample:

$$\frac{\text{Glycogen content}}{(\text{mg/g tissue})} = \frac{\Delta A_1}{\Delta A_2} \times m \times f \times 10* \div 1.11*$$

[Note]

ΔA_1: $OD_{Sample} - OD_{Blank}$.

ΔA_2: $OD_{Standard} - OD_{Blank}$.

m: The content of standard (0.01 mg).

f: Dilution factor of sample in sample preparation. For example, 1% liver glycogen testing solution, f = 100; 5% muscle glycogen testing solution, f = 20.

10*: The dilution factor of reaction system.

1.11*: The coefficient for converting the glycose concentration to glycogen concentration. The color degree of 100 μg of glycogen developed with anthracenone is equal to 111 μg of glycose with same treatment.

Appendix I Performance Characteristics

1. Parameter:

Intra-assay Precision

Three mouse liver tissue 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 (mg/g)	5.80	16.70	45.60
%CV	3.9	3.7	3.5

Inter-assay Precision

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

Parameters	Sample 1	Sample 2	Sample 3
Mean (mg/g)	5.80	16.70	45.60
%CV	6.9	7.5	7.5

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 101%.

	Sample 1	Sample 2	Sample 3
Expected Conc. （mg/g）	26.4	78.6	154.5
Observed Conc. （mg/g）	26.7	81.0	153.0
recovery rate(%)	101	103	99

Sensitivity

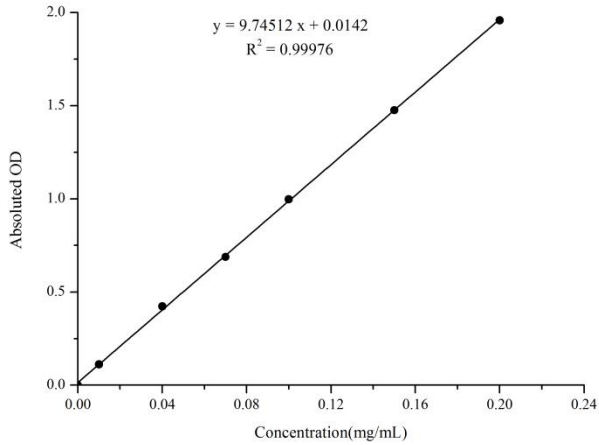
The analytical sensitivity of the assay is 1.8 mg/g liver tissue or 0.36 mg/g muscle tissue. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 20 times, and calculating the corresponding concentration.

2. Standard curve:

(It doesn't need to prepare the standard curve for this kit and the provided standard curve is for reference only)

As the OD 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 (mg/mL)	**0**	**0.01**	**0.04**	**0.07**	**0.1**	**0.15**	**0.2**
Average OD	0.058	0.170	0.450	0.746	1.055	1.534	2.016
Absoluted OD	0	0.112	0.392	0.688	0.997	1.476	1.958



Appendix Π Example Analysis

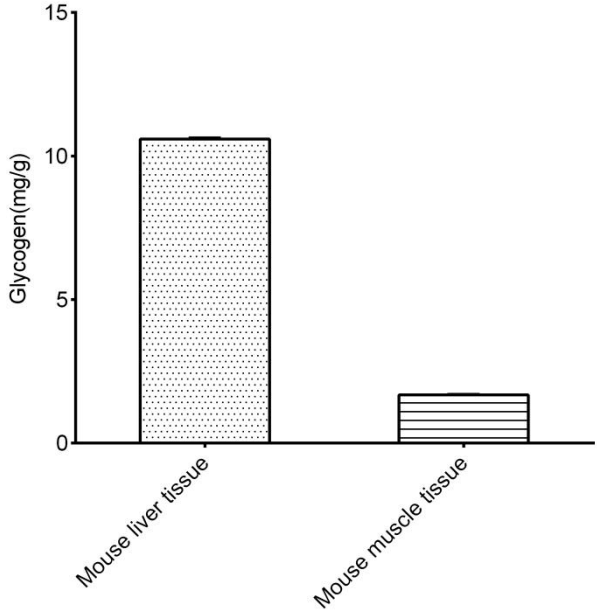
Example analysis：

For mouse liver tissue, carry the assay according to the operation steps. The results are as follows:

The average OD value of the sample is 0.358, the average OD value of the blank is 0.181, the average OD value of the standard is 0.331, and the calculation result is:

$$\text{Glycogen content (mg/g tissue)} = \frac{0.358-0.181}{0.331-0.181} \times 0.01 \times 100 \times 10 \div 1.11 = 10.63 \text{ mg/g tissue}$$

Detect mouse liver tissue (1% liver glycogen testing solution), mouse muscle tissue (5% muscle glycogen testing solution) 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.