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

Catalog No：E-BC-K340-M

Specification：48T(32 samples)/96T(80 samples)

Measuring instrument：Microplate reader（500-520 nm）

Detection range：0.01-1.00 mmol/L

Elabscience® Glycerol Colorimetric Assay Kit

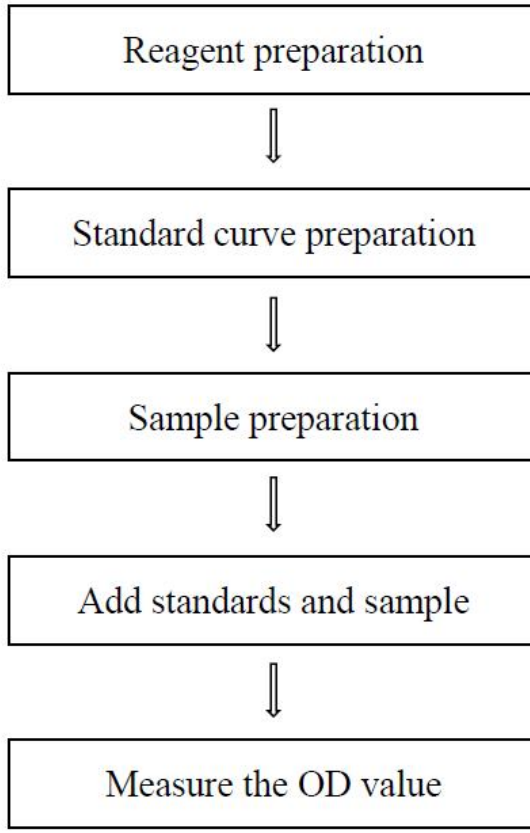
This manual must be read attentively and completely before using this product.
If you have any problem, please contact our Technical Service Center for help：

Toll-free: 1-888-852-8623
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.

Table of contents

Assay summary



Intended use

This kit can be used to measure glycerol in serum (plasma), animal tissue and cell samples.

Detection principle

Glycerol is an intermediate product of triglyceride metabolism in animal and plant tissues and blood. Glycerol is hydrolyzed to produce glycerol, which is further oxidized to provide energy for cell metabolism. Therefore, glycerol content is a reliable indicator of triglyceride hydrolysis reaction and detection is more convenient.

Glycerol is transformed by enzyme to produce hydrogen peroxide, which is catalyzed by oxidase in the presence of 4-amino-antipyrprid and phenol to produce red quinones, the color of which is proportional to the content of glycerol.

Kit components & storage

Item	Component	Size 1(48 T)	Size 2(96 T)	Storage
Reagent 1	Working Solution	12.5 mL × 1 vial	25 mL × 1 vial	2-8℃, 12 months shading light
Reagent 2	1.0 mmol/L Standard	1 mL × 1 vial	1 mL × 2 vials	2-8℃, 12 months shading light
	Microplate	48 wells	96 wells	No requirement
	Plate Sealer	2 pieces		

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:

Water bath, Microplate reader (500-520 nm, optimum wavelength: 510 nm)

Reagents:

Double distilled water, Isopropanol (AR), PBS(0.01 M，pH 7.4)

Reagent preparation

① Equilibrate all the reagents to room temperature before use.

② The preparation of standard curve：

Always prepare a fresh set of standards. Discard working standard dilutions after use.

Dilute 1 mmol/L standard solution with double distilled water to a serial concentration. The recommended dilution gradient is as follows: 0.0, 0.1, 0.2, 0.4, 0.5, 0.6, 0.8, 1.0 mmol/L. Reference is as follows:

Item	①	②	③	④	⑤	⑥	⑦	⑧
Concentration (mmol/L)	**0**	**0.1**	**0.2**	**0.4**	**0.5**	**0.6**	**0.8**	**1.0**
1.0 mmol/L copper standard (μL)	0	20	40	80	100	120	160	200
Double distilled water (μL)	200	180	160	120	100	80	40	0

Sample preparation

① Sample preparation

Serum and plasma: detect directly. If not detected on the same day, the serum or plasma can be stored at -80℃ for a month.

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 isopropanol with a dounce homogenizer at 4℃.

④ Then inactivated by water bath at 70°C for 10 min, centrifuge at 10000×g for 10 min, take the supernatant for detection.

Cells:

① Harvest the number of cells needed for each assay (initial recommendation 2×10^6 cells).

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

③ Homogenize 2×10^6 cells in 100 μL isopropanol with sonicate or mechanical homogenate at 4°C.

④ Then inactivated by water bath at 70°C for 10 min, centrifuge at 10000×g for 10 min, take the supernatant for detection.

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	2
10% Mouse kidney tissue homogenate	1
10% Mouse spleen tissue homogenate	1
Cell	1

Note: The diluent is double distilled water. For the dilution of other sample types, please do pretest to confirm the dilution factor

The key points of the assay

① Touch the bottom of the plate when adding standards and samples.

② There should be no bubbles in the wells of the microplate when measuring the OD value.

Operating steps

① Standard well: Add 10 μL of standard solution with different concentrations to the corresponding wells.

Sample well: Add 10 μL of sample to the corresponding wells.

② Add 250 μL of working solution to each well..

③ Mix fully with microplate reader for 5 s and incubate at 37°C for 10 min. Measure the OD value of sample well at 510 nm with microplate reader.

Calculation

The standard curve:

1. Average the duplicate reading for each standard.

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

3. Plot the standard curve by using absoluted OD 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. Serum (plasma) sample:

$$\underset{(\text{mmol/L})}{\text{glycerol content}} = (\Delta A_{510} - b) \div a \times f$$

2. Tissue sample:

$$\underset{(\text{mmol/kg wet weight})}{\text{glycerol content}} = (\Delta A_{510} - b) \div a \times V \div m \times f$$

3. Cell sample:

$$\underset{(\mu\text{mol/10\^6})}{\text{glycerol content}} = (\Delta A_{510} - b) \div a \times V \div N \times f$$

[Note]

ΔA_{510}: OD_{Sample} – OD_{Blank} (OD_{Blank} is the OD value when the standard concentration is 0).

V: The volume of isopropanol, mL.

m: The weight of sample, g.

N: The number of cells, 10^6.

f: Dilution factor of sample before test.

Appendix I Performance Characteristics

1. Parameter:

Intra-assay Precision

Three human serum 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 (mmol/L)	0.25	0.64	0.82
%CV	4.2	3.9	3.9

Inter-assay Precision

Three human serum samples were assayed 17 times in duplicate by three operators to determine precision between assays.

Parameters	Sample 1	Sample 2	Sample 3
Mean (mmol/L)	0.25	0.64	0.82
%CV	8.2	7.8	8.0

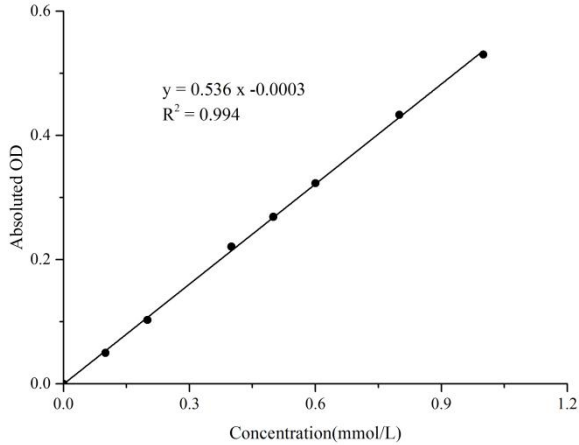
Sensitivity

The analytical sensitivity of the assay is 0.01mmol/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 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 (mmol/L)	**0.00**	**0.10**	**0.20**	**0.40**	**0.50**	**0.60**	**0.80**	**1.00**
Average OD	0.072	0.122	0.175	0.293	0.340	0.394	0.505	0.601
Absoluted OD	0.000	0.050	0.103	0.221	0.269	0.323	0.433	0.530



Appendix II Example Analysis

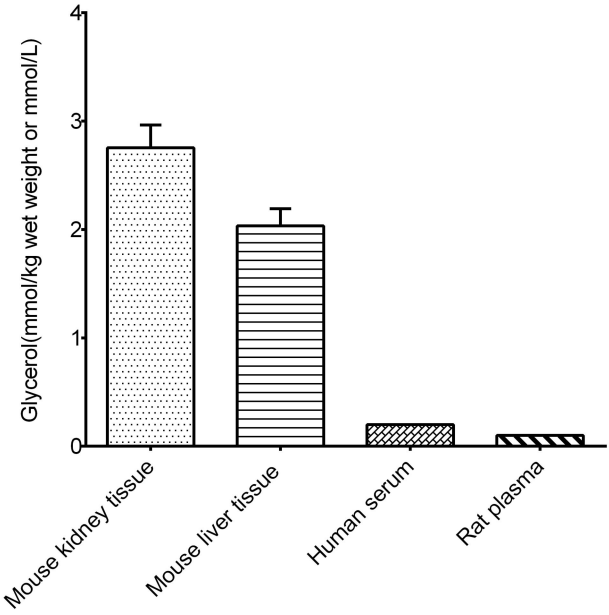
Example analysis:

For mouse kidney tissue, take 10 μL of 10% mouse liver tissue homogenate, carry the assay according to the operation steps. The results are as follows:

standard curve: y = 0.536 x – 0.003, the OD value of the blank is 0.072, the OD value of the sample is 0.244, and the calculation result is:

glycerol content (mmol/kg wet weight) = (0.244 - 0.072 + 0.003) ÷ 0.536 × 0.9 ÷ 0.1 = 2.93 mmol/kg wet weight

Detect 10% mouse kidney tissue homogenate, 10% mouse liver tissue homogenate, human serum and rat plasma 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.