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

Catalog No：E-BC-F065

Specification：96T(84 samples)

Measuring instrument：Fluorescence Microplate Reader

(Ex/Em=535 nm/587 nm)

Detection range：1.4-32 μmol/L

Elabscience® Adipogenesis 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.

Table of contents

Assay summary 3

Intended use 4

Detection principle 4

Kit components & storage 4

Materials prepared by users 5

Reagent preparation 5

Sample preparation 6

The key points of the assay 7

Operating steps 8

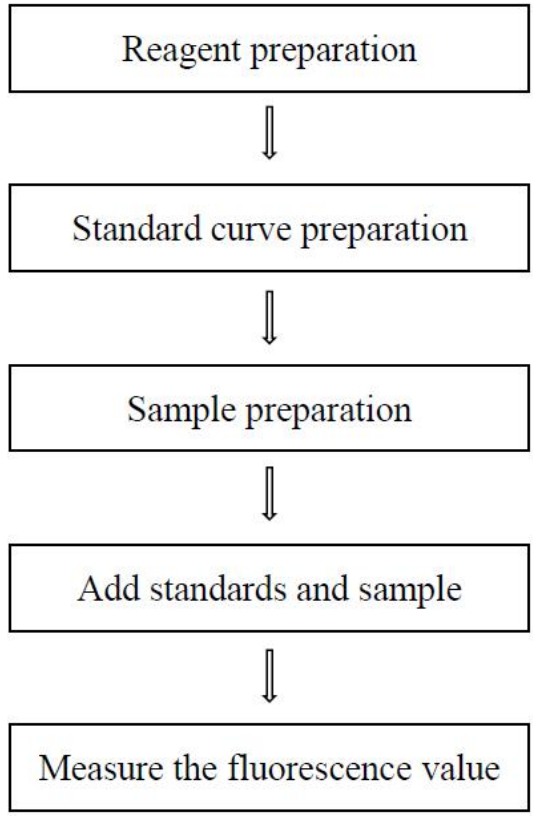
Calculation 9

Appendix I Performance Characteristics 10

Appendix II Example Analysis 12

Statement 13

Assay summary



Intended use

This kit can be used to measure the choline/acetylcholine content in tissue and cell samples.

Detection principle

Triglycerides were converted by enzyme to produce hydrolytic products, which were catalyzed by the enzyme to produce fluorescent substances. The triglyceride accumulation can be calculated by meas-uring the fluorescence intensity at the excitation wavelength of 535 nm and the emission wavelength of 587 nm.

Kit components & storage

Item	Component	Size (96 T)	Storage
Reagent 1	Enzyme Working Solution	25 mL × 1 vial	2-8℃, 12 months shading light
Reagent 2	Extraction Solution	50 mL × 1 vial	2-8℃, 12 months
Reagent 3	Probe	0.5 mL × 1 vial	-20℃, 12 months shading light
Reagent 4	1 mmol/L Standard	0.5 mL × 1 vial	2-8℃, 12 months shading light
	Black Microplate	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:

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 chromogenic working solution:

For each well, prepare 200 μL of chromogenic working solution (mix well 196 μL of enzyme working solution and 4 μL of probe).The chromogenic working solution should be prepared on spot and protected from light.

③ The preparation of 40 μmol/L standard solution:

Dilute 20 μL of 1 mmol/L standard solution with 480 μL of extraction solution and mix fully. Store at 2-8°C for 3 days protected from light.

④ The preparation of standard curve：

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

Dilute 40 μmol/L standard with extraction solution to a serial concentration.

The recommended dilution gradient is as follows: 0, 8, 16, 20, 24, 32 μmol/L.

Reference is as follows:

Item	①	②	③	④	⑤	⑥
Concentration (μmol/L)	**0**	**8**	**16**	**20**	**24**	**32**
40 μmo/L standard (μL)	0	40	80	100	120	160
Extraction solution (μL)	200	160	120	100	80	40

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 extraction solution with a dounce homogenizer at 4℃.

④ Centrifuge at 10000×g for 10 min to remove insoluble material. Collect supernatant and keep it on ice for detection.

⑤ Meanwhile, determine the protein concentration of supernatant (E-BC-K318-M).

⑥ Place on ice for 30 min.

⑦ Heat the supernatant at 90-100°C for 30 min to ensure the lipids completely dissolved in extraction solution. Cool to room temperature for detection. If there is turbidity, it is recommended to centrifuge again and take the supernatant for determination. (The lipid content of the tissue sample is relatively high, so it is recommended to take 2~3 samples to do a pre-experiment and choose an appropriate dilution ratio for dilution. The diluent is extraction solution).

Cell (adherent or suspension) samples:

① Harvest the number of cells needed for each assay (initial recommendation 1×10^4 cells).

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

③ Homogenize 1×10^4 cells in 200 μL extraction solution with a ultrasonic cell disruptor at 4℃.

④ Centrifuge at 10000×g for 10 min to remove insoluble material. Collect supernatant and keep it on ice for detection.

⑤ Meanwhile, determine the protein concentration of supernatant (E-BC-K318-M).

⑥ Heat the supernatant at 90-100°C for 30 min to ensure the lipids completely dissolved in extraction solution. Cool to room temperature for detection.

② Dilution of sample

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

Sample type	Dilution factor
10% Porcine heart tissue homogenate	3-6
10% Porcine liver tissue homogenate	8-10
CHO cell (10^6)	1
293T cell (10^6)	1

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

The key points of the assay

It is necessary to wash the preparation equipment several times before preparation of the chromogenic working solution to prevent impurity contamination.

Operating steps

① Standard well: add 20 μL of standard with different concentrations to the corresponding wells.

Sample well: add 20 μL of sample supernatant to the corresponding wells.

② Add 200 μL chromogenic working solution to each well.

③ Mix well and incubate at 37°C for 5 min.

④ Measure the fluorescence intensity of each well at the excitation wavelength of 535 nm and the emission wavelength of 587 nm.

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:

Tissue and cell samples:

$$\text{Triglyceride content (μmol/gprot)} = (\Delta F - b) \div a \div C_{pr} \times f$$

[Note]

ΔF: $F_{Sample} - F_{Blank}$. (F_{Blank} is the fluorescence value when the standard concentration is 0)

C_{pr}: Concentration of protein in sample, gprot/L.

f: Dilution factor of sample before test.

Appendix I Performance Characteristics

1. Parameter:

Intra-assay Precision

Three porcine heart 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 (μmol/L)	2.00	15.00	25.00
%CV	4.3	3.8	3.9

Inter-assay Precision

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

Parameters	Sample 1	Sample 2	Sample 3
Mean (μmol/L)	2.00	15.00	25.00
%CV	9.7	7.9	7.6

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 98%.

	Standard 1	Standard 2	Standard 3
Expected Conc. (μmol/L)	8	20	25
Observed Conc. (μmol/L)	8.0	19.6	24.0
Recovery rate (%)	100	98	96

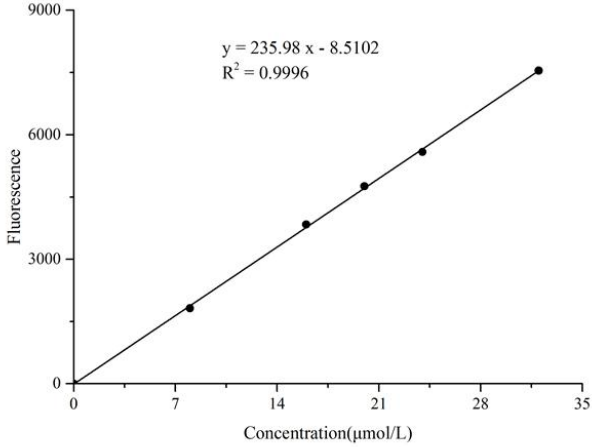
Sensitivity

The analytical sensitivity of the assay is 1.4 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 (μmol/L)	**0**	**8**	**16**	**20**	**24**	**32**
Fluorescence value	2488	4332	6347	7177	8215	10135
	2395	4189	6213	7226	7840	9835
Average fluorescence value	2441.5	4260.5	6280	7201.5	8027.5	9985
Absoluted fluorescence value	0	1819	3838.5	4760	5586	7543.5



Appendix II Example Analysis

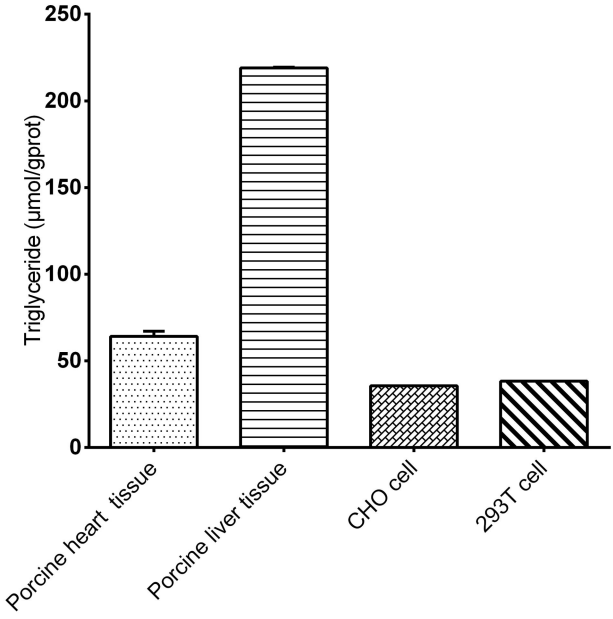
Example analysis：

For porcine heart tissue, take 10% tissue homogenate supernatant, dilute for 5 times, then carry the assay according to the operation steps. The results are as follows:

standard curve: y = 235.98 x - 8.5102, the average fluorescence value of the sample well is 9614, the average fluorescence value of the blank well is 2121, the concentration of protein is 2.56 gprot/L and the calculation result is:

Triglyceride content (μmol/gprot) = (9614- 2121+ 8.5102) ÷ 235.98 ÷ 2.56 × 5= 62.08 μmol/gprot

Detect 10% porcine heart tissue homogenate (the concentration of protein is 2.56 gprot/L, dilute for 5 time), 10% porcine liver tissue homogenate (the concentration of protein is 5.67 gprot/L, dilute for 5 time), CHO cell (the concentration of protein is 0.98 gprot/L) and 293T cell (the concentration of protein is 1.21 gprot/L) 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.