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

Catalog No：E-BC-K798-M

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

Measuring instrument：Microplate reader(535-575 nm)

Detection range：0.005-0.543 mmol/L

Elabscience® Lipolysis 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.

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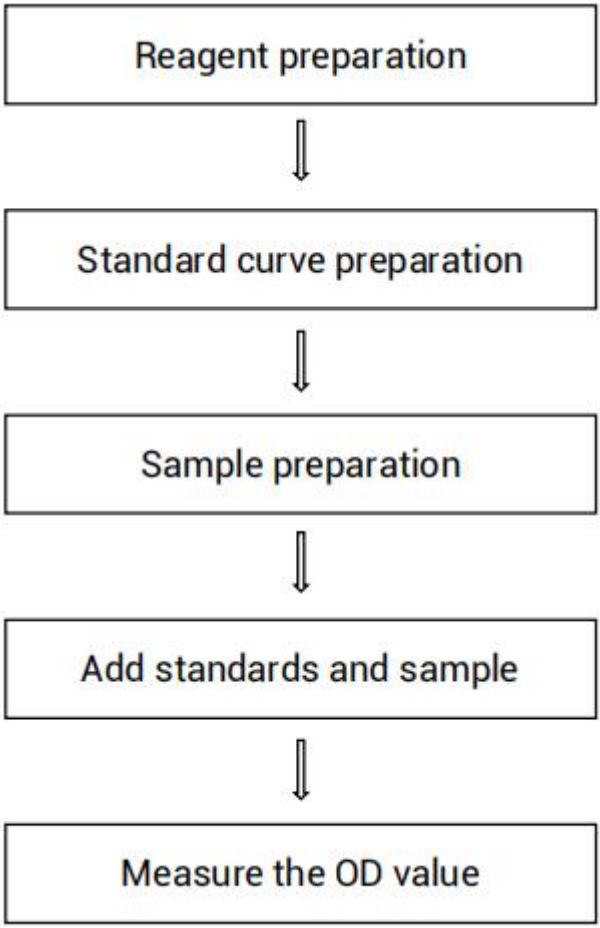
Assay summary



Intended use

This kit can be used to measure lipolysis level in cell samples.

Detection principle

Lipolysis is the process by which the body converts fat reserves into energy, the fat stored in fat cells is gradually hydrolyzed under the catalysis of lipase, releasing free fatty acid (FFA) and glycerin (Gly). The glycerol produced by lipolysis cannot be used by the cells themselves, and needs to be transported to the liver for further conversion into energy. The content of glycerol can reflect the level of lipolysis in the cell sample.
The detection principle of this kit is to make the glycerol released by cells produce hydrogen peroxide under the catalysis of lipase, and hydrogen peroxide reacts with enzymes and chemical substances to produce specific products, which have maximum absorption at 555 nm, and OD value is proportional to the concentration within a certain range.

Kit components & storage

Item	Component	Size 1(48 T)	Size 2(96 T)	Storage
Reagent 1	Assay Buffer	30 mL × 1 vial	55 mL × 1 vial	2-8 ℃ , 12 months
Reagent 2	Matrix Solution	9 mL × 1 vial	16.5 mL × 1 vial	2-8 ℃, 12 months, shading light
Reagent 3	Chromogenic Agent	0.9 mL × 1 vial	1.65 mL × 1 vial	2-8 ℃, 12 months, shading light
Reagent 4	10mmol/L Standard	0.24 mL × 1 vial	0.24 mL × 1 vial	2-8 ℃, 12 months, shading light
	Microplate	48 wells	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:

Microplate reader (535-575 nm, optimum wavelength: 555 nm), Incubator, Vortex mixer

Reagent:

Normal saline (0.9% NaCl)

Reagent preparation

① Equilibrate all reagents to 25℃ before use.

② The preparation of chromogenic working solution：

Before testing, please sufficient prepare chromogenic working solution according to the test wells. For example, prepare 165 μL of chromogenic working solution (mix well 150 μL of matrix solution and 15 μL of chromogenic agent). Store at 2-8°C for 3 days protected from light.

③ The preparation of 0.5 mmol/L standard solution：

Before testing, please prepare sufficient 0.5 mmol/L standard solution. For example, prepare 1000 μL of 0.5 mmol/L standard solution (mix well 50 μL of 10 mmol/L standard and 950 μL of double distilled water). Store at 2-8°C for a week protected from light.

④ The preparation of standard curve：

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

Dilute 0.5 mmol/L standard solution with double distilled water to a serial concentration. The recommended dilution gradient is as follows: 0, 0.1, 0.2, 0.25, 0.3, 0.35, 0.4, 0.5 mmol/L. Reference is as follows:

Item	①	②	③	④	⑤	⑥	⑦	⑧
Concentration (mmol/L)	0	0.1	0.2	0.25	0.3	0.35	0.4	0.5
0.5 mmol/L standard (μL)	0	40	80	100	120	140	160	200
Double distilled water (μL)	200	160	120	100	80	60	40	0

Operating steps

Cell samples: Cell culture or differentition is performed according to the experimental design to ensure that the cells are healthy and do not overgrow. After cell culture, remove culture medium, and wash them with assay buffer for twice. For each washing, remove assay buffer slowly.

① Add appropriate amount of assay buffer and mix well gently. Add 1-2 μL of drug stimulation according to the experimental requirements, and set up the corresponding control group. (Assay buffer can maintain the growth of cells within 8 hours. If the drug stimulation time is longer, it is recommended to use serum-free medium for culture).

② Incubate and collect the cell supernatant for testing.

③ Standard well: add 20 μL of different concentrations of standard solutions to the wells.

Sample well: add 20 μL of cell suspension to the wells.

④ Add 150 μL of chromogenic working solution to the standard well and the sample well.

⑤ Incubate at 25°C for 10 min, measure the OD value of each well at 555 nm with microplate reader.

Note: Determine the protein concentration of supernatant (E-BC-K318-M).

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:

For cell samples:

$$\text{lipolysis (mmol/gprot)} = \frac{\Delta A - b}{a} \div C_{pr}$$

[Note]

ΔA: The OD values of sample well - The OD values of blank well (The OD value when the standard concentration is 0)

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

Appendix I Performance Characteristics

1. Parameter:

Intra-assay Precision

Three 293T cell 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)	0.15	0.27	0.37
%CV	1.4	1.3	1.4

Inter-assay Precision

Three 293T cell samples were assayed 20 times in duplicate by three operators to determine precision between assays.

Parameters	Sample 1	Sample 2	Sample 3
Mean (mmol/L)	0.15	0.27	0.37
%CV	1.6	2.2	2.5

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 99%.

	Standard 1	Standard 2	Standard 3
Expected Conc. (mmol/L)	0.15	0.27	0.37
Observed Conc. (mmol/L)	0.1	0.3	0.4
Recovery rate (%)	98	100	99

Sensitivity

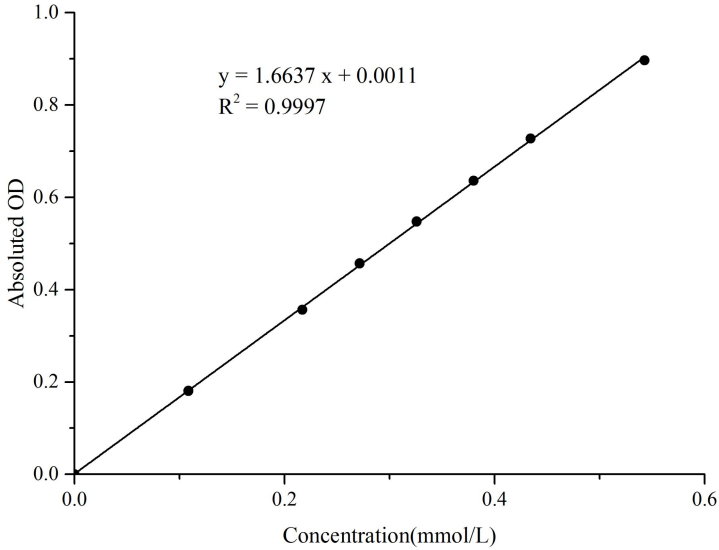
The analytical sensitivity of the assay is 0.005 mmol/L. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 20 times, and calculating the

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	0.1	0.2	0.25	0.3	0.35	0.4	0.5
OD	0.146	0.329	0.504	0.597	0.691	0.782	0.871	1.045
	0.146	0.324	0.501	0.609	0.696	0.782	0.876	1.041
Average OD	0.146	0.327	0.503	0.603	0.694	0.782	0.874	1.043
Absoluted OD	0	0.181	0.357	0.457	0.548	0.636	0.728	0.897



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.