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

Catalog No：E-BC-K784-M

Specification：96T(40 samples)

Measuring instrument：Microplate reader(440-460 nm)

Detection range：0.10-16.67 U/L

Elabscience® Fatty Acid Oxidation (FAO) 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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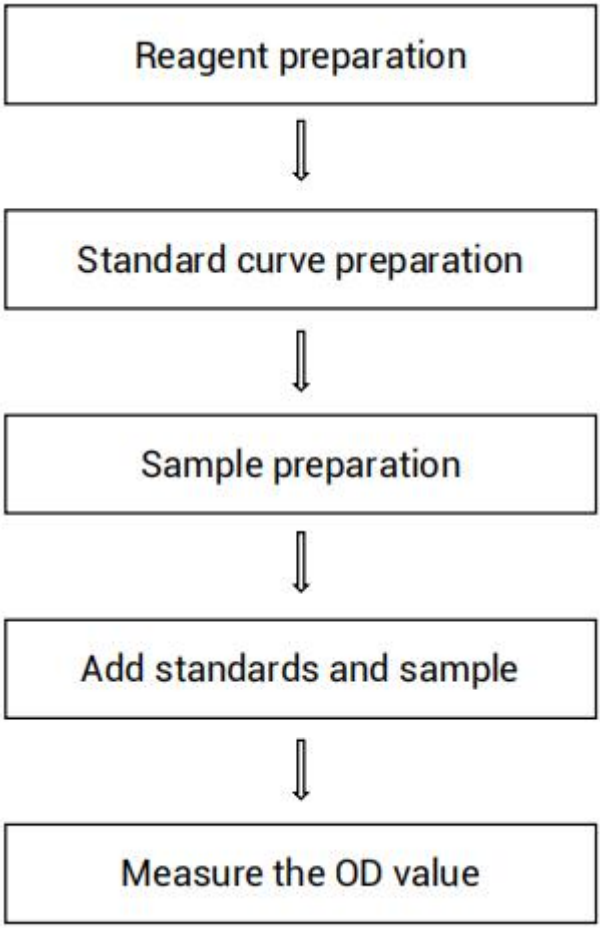
Assay summary



Intended use

The kit is suitable for detecting the fatty acid oxidation (FAO) ability in animal tissues and cells.

Detection principle

Fatty acid oxidation (FAO) is the main pathway of fatty acid decomposition in the body, which can supply a large amount of energy required by the organism. FAO is also the transformation process of fatty acids. The length of fatty acid chain required by the organism is different. Through oxidation, long-chain fatty acids can be transformed into fatty acids with suitable growth degree for the organism metabolism.

FAO process consumes the fatty acid substrate and NAD^+, and generates NADH. Under the action of electron coupling agent and chromogenic agent, the orange red substance is generated, which can be detected at 450 nm.

Kit components & storage

Item	Component	Size (96 T)	Storage
Reagent 1	Extraction Solution	0.55 mL×1vial	-20°C, 12 months, shading light
Reagent 2	Buffer Solution	40 mL × 2 vials	-20°C, 12 months, shading light
Reagent 3	Co-factor	Powder ×2 vials	-20°C, 12 months, shading light
Reagent 4	Substrate	0.44 mL ×1 vial	-20°C, 12 months, shading light
Reagent 5	Chromogenic Agent	1.2 mL ×2 vials	-20°C, 12 months, shading light
Reagent 6	Standard	Powder ×2 vials	-20°C, 12 months, shading light
	Microplate	96 wells	No requirement
	Plate Sealer	2 pieces	
	Sample Layout Sheet	1 piece	

Note: All the reagents should be stored according to the table. The reagents from different kits can not be mixed or used interchangeably. For liquid reagents with small volumes or powders, centrifuge them before use to prevent loss.

Instruments

Microplate reader (440-460 nm, optimum wavelength: 450 nm), Incubator (37°C)

Materials required but not provided

Double distilled water, 1×PBS (0.01 M, pH 7.4)

Reagent preparation

① Equilibrate all the reagents to 25°C before use.

② Extraction working solution preparation：

Before testing, please prepare sufficient extraction working solution according to the number of samples. For example, prepare 1010 μL of extraction working solution (mix well 10 μL of extraction solution and 1000 μL of buffer solution).The extraction working solution should be prepared on spot. Keep it on ice during use protected from light and used up within 8 h.

③ Co-factor working solution preparation：

Dissolve one vial of co-factor with 1 mL of buffer solution, mix well to dissolve. Keep it on ice during use protected from light. It is stable for 3 days when stored at -20°C.

④ Substrate working solution Preparation：

Before testing, please prepare sufficient substrate working solution

according to the test wells. For example, prepare 50 μL of substrate working solution (mix well 40 μL of buffer solution and 10 μL of substrate). The substrate working solution should be prepared on spot and keep it on ice during use protected from light. It is stable for 3 days when stored at -20°C.

⑤ Chromogenic working solution preparation：

For each well, prepare 171 μL of chromogenic working solution (mix well 145 μL of buffer solution, 5 μL of co-factor working solution and 21 μL chromogenic agent). The chromogenic working solution should be prepared on spot. Keep it on ice during use protected from light and used up with in 8 h..

⑥ 0.5 mmol/L standard solution preparation：

Dissolve one vial of standard with 5 mL of double distilled water, mix well to dissolve. Keep it on ice during use protected from light. It is stable for 3 days when stored at -20°C.

⑦ Standard curve preparation：

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, 0.15, 0.2, 0.3, 0.4, 0.45, 0.5 mmol/L.

Reference is as follows:

Item	①	②	③	④	⑤	⑥	⑦	⑧
Concentration (mmol/L)	**0**	**0.1**	**0.15**	**0.2**	**0.3**	**0.4**	**0.45**	**0.5**
0.5 mmol/L standard (μL)	0	40	60	80	120	160	180	200
Double distilled water (μL)	200	160	140	120	80	40	20	0

Sample preparation

Tissue sample:

① Harvest the amount of tissue needed for each assay (initial recommendation 30 mg).

② Wash tissue in 1×PBS (0.01 M, pH 7.4).

③ Homogenize 30 mg tissue in 270 μL extraction working solution with a dounce homogenizer at 4°C.

④ Centrifuge at 10000×g for 15 min to remove insoluble material. Collect supernatant and keep it on ice for detection. Detect the prepared tissue supernatant within 4 h.

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

Cell sample:

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

② Wash cells with 1×PBS (0.01 M, pH 7.4).

③ Homogenize 1×10^6 cells in 200 μL extraction working solution with a ultrasonic cell disruptor at 4°C.

④ Centrifuge at 10000 × g for 15 min at 4°C to remove insoluble material. Collect supernatant and keep it on ice for detection. Detect the prepared tissue supernatant within 4 h.

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

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	2-4
10% Mouse spleen tissue homogenate	1
10% Mouse kidney tissue homogenate	2-4
10% Mouse heart tissue homogenate	2-4
1×10^6 K562 cells	1
1×10^6A549 cells	1
1×10^6 Jurkat cells	1
1×10^6 293T cells	1

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

The key points of the assay

The initial OD value A_1 is used exclusively to assess whether sample dilution is necessary, where an A_1 value >0.5 in the control well indicates that dilution is required.

Operating steps

① Standard well: Add 30 μL of standard solution with different concentrations to the corresponding wells.
Sample well: Add 30 μL of sample to the corresponding wells.
Control well: Add 30 μL of sample to the corresponding wells.

② Add 165 μL of chromogenic working solution to each well,

③ add 40 μL of substrate working solution to sample wells and add 40 μL of buffer solution to control and standard wells.

④ Mix fully with microplate reader for 5 s .Measure the initial OD value (A1) of each well at 450 nm with microplate reader.

⑤ Incubate at 37°C for 30 min. Measure the OD value (A_2) of each well at 450 nm with microplate reader. (The standard curve is fitted to the standard well in A_2 value. If the initial A_1 of the control well > 0.5, the sample should be diluted. If the ΔA_{450} <0.005, the reaction time may be extended, with a corresponding adjustment made to the time parameter in the calculation formula.)

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:

Tissue and cell samples:

Definition: The amount of enzyme in 1 g protein per 1 minute that hydrolyze the substrate to produce 1 μmol NADH at 37°C is defined as 1 unit.

$$\text{FAO ability(U/gprot)} = (\Delta A_{450} - b) \div a \div T \times 1000 \div C_{pr} \times f$$

[Note]

ΔA_{450}: $A_{2\ Sample} - A_{2\ Control}$.

T: Reaction time, 30 min.

1000*: 1 mmol/L = 1000 μmol/L.

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

f: Dilution factor of sample before test.

Appendix I Performance Characteristics

1. Parameter:

Intra-assay Precision

Three mouse liver 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 (U/L)	1.00	3.00	6.00
%CV	4.1	4.8	5.0

Inter-assay Precision

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

Parameters	Sample 1	Sample 2	Sample 3
Mean (U/L)	1.00	3.00	6.00
%CV	8.2	9.1	9.4

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 102%.

	Sample 1	Sample 2	Sample 3
Expected Conc. (U/L)	1.00	3.00	6.00
Observed Conc. (U/L)	1.0	3.0	6.6
Recovery rate (%)	103	100	103

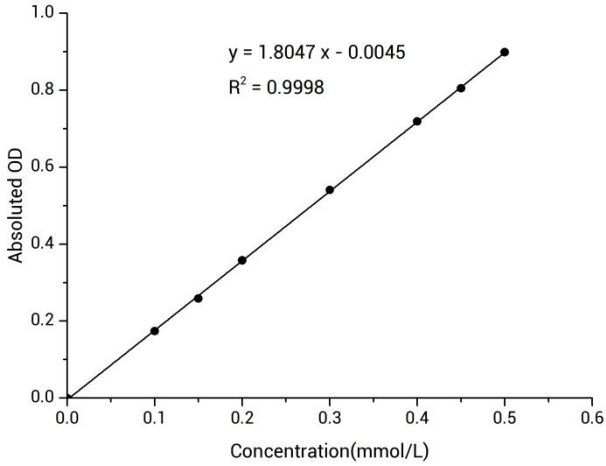
Sensitivity

The analytical sensitivity of the assay is 0.10 U/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	0.15	0.2	0.3	0.4	0.45	0.5
OD	0.057	0.229	0.316	0.416	0.596	0.773	0.851	0.953
	0.057	0.232	0.316	0.414	0.600	0.779	0.872	0.959
Average OD	0.057	0.231	0.316	0.415	0.598	0.776	0.862	0.956
Absoluted OD	0	0.174	0.259	0.358	0.541	0.719	0.805	0.899



Appendix II Example Analysis

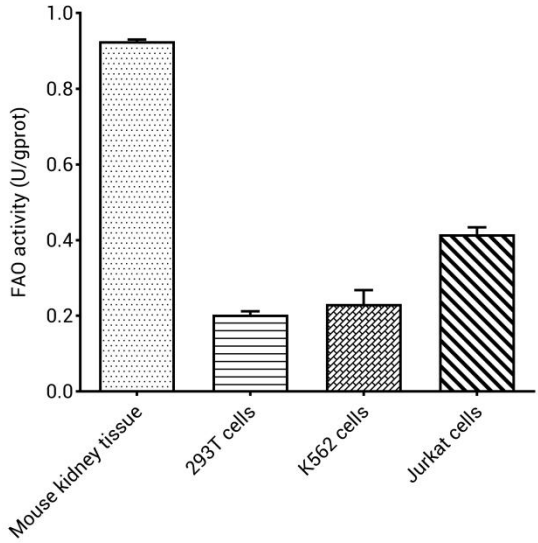
Example analysis：

Take 30 μL of 2-fold diluted 10% mouse kidney tissue homogenate. Proceed according to the operating steps.The results are as follows:

standard curve: y = 1.8047 x - 0.0045, the A_2 of the sample well is 1.132, the A_2 of the control well is 0.823, ΔA_{450} = 1.132 - 0.723 = 0.309, the concentration of protein is 12.53 gprot/L, and the calculation result is:

FAO ability (U/gprot)= (0.309 + 0.0045) ÷ 1.8047 ÷ 30 × 2 × 1000 ÷ 12.53 = 0.924 U/gprot

Detect 10% mouse kidney tissue homogenate (the concentration of protein is 12.53 gprot/L), 1×10^6 293T cells (the concentration of protein is 0.48 gprot/L), 1×10^6 K562 cells (the concentration of protein is 0.33 gprot/L), 1×10^6 jurkat cells (the concentration of protein is 0.56 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.