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

Catalog No：E-BC-F077

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

Measuring instrument：Fluorescence Microplate Reader

(Ex/Em=507 nm/547 nm)

Detection range：0.05-0.50 U/L

Elabscience® Lipoxygenase (LOX) Activity 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

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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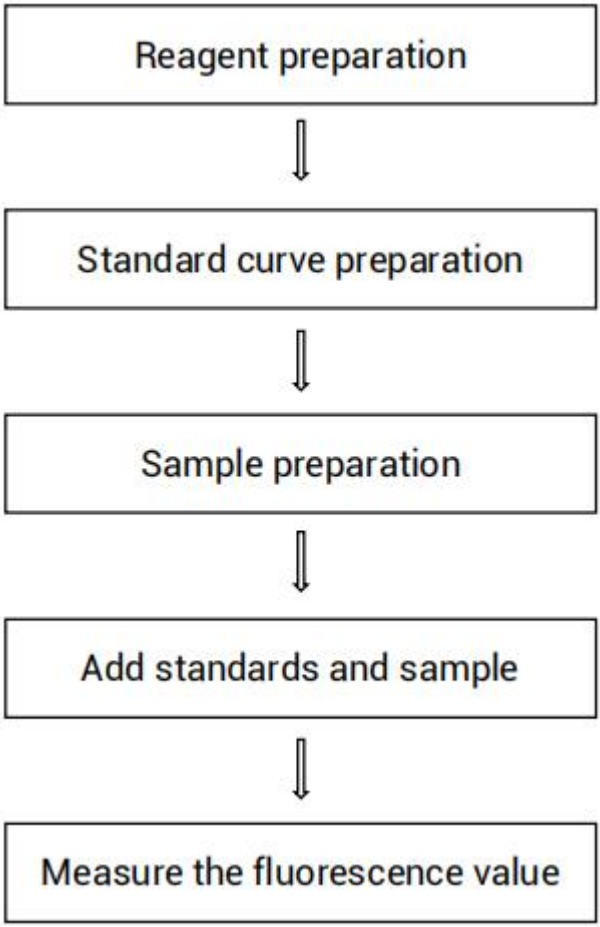
Assay summary



Intended use

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

Detection principle

Lipoxygenase (LOX) is an iron-containing oxidoreductase found in plants and animals. The detection principle of this kit is as follows: LOX catalyzes the oxidation of arachidonic acid to produce fluorescence products, and the enzyme activity of LOX can be calculated according to the change of fluorescence value of fluorescence products at the excitation wavelength of 507 nm and the emission wavelength of 547 nm within a unit time.

Kit components & storage

Item	Component	Size 1(48 T)	Size 2(96 T)	Storage
Reagent 1	Buffer Solution	7 mL × 1 vial	14 mL × 1 vial	-20°C, 12 months shading light
Reagent 2	Substrate	0.7 mL × 1 vial	1.4 mL × 1 vial	-20°C, 12 months shading light
Reagent 3	Probe	Powder × 1 vial	Powder × 2 vials	-20°C, 12 months shading light
Reagent 4	Probe Solution	0.15 mL × 1 vial	0.3 mL × 1 vial	-20°C, 12 months shading light
Reagent 5	1 mmol/L Standard	0.1 mL × 1 vial	0.1 mL × 1 vial	-20°C, 12 months shading light
	Black Microplate	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:

Fluorescence microplate reader (Ex/Em=507 nm/547 nm), Incubator (37℃)

Reagents:

Normal saline (0.9% NaCl)

Reagent preparation

① Equilibrate all reagents to 25℃ before use.

② The preparation of substrate working solution:
Before testing, please prepare sufficient substrate working solution. For example, prepare 550 μL of substrate working solution (mix well 500 μL of buffer solution and 50 μL of substrate). The substrate working solution should be prepared on spot and used up within 1 h.

③ The preparation of probe working solution:
Dissolve one vial of probe with 0.09 mL of probe solution, mix well to dissolve. Store at -20°C for 2 weeks protected from light.

④ The preparation of reaction working solution:
Before testing, please prepare sufficient reaction working solution according to test wells. For example, prepare 505 μL of reaction working solution (mix well 500 μL of substrate working solution and 5 μL of probe working solution). The reaction working solution should be prepared on spot and used up within 1 h.

⑤ The preparation of 10 μmol/L standard solution:

Before testing, please prepare sufficient 10 μmol/L standard solution. For example, prepare 1000 μL of 10 μmol/L standard solution (mix well 10 μL of 1 mmol/L standard and 990 μL of double distilled water). Store at -20°C for 2 weeks protected from light.

⑥ The preparation of standard curve:

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

Dilute 10 μmol/L standard solution with double distilled water to a serial concentration. The recommended dilution gradient is as follows: 0, 2, 4, 5, 6, 7, 8, 10 μmol/L. Reference is as follows:

Item	①	②	③	④	⑤	⑥	⑦	⑧
Concentration (μmol/L)	**0**	**2**	**4**	**5**	**6**	**7**	**8**	**10**
10 μmol/L Standard (μL)	0	40	80	100	120	140	160	200
Double distilled water (μL)	200	160	120	100	80	60	40	0

Sample preparation

① Sample preparation

Serum (plasma) samples: detect directly.

Tissue samples:

① Harvest the amount of tissue needed for each assay (initial recommendation 20 mg).

② Wash tissue in normal saline (0.9% NaCl).

③ Homogenize 20 mg tissue in 180 μL normal saline (0.9% NaCl) with a dounce homogenizer at 4℃.

④ Centrifuge at 10000×g for 10 min at 4℃ 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).

Cell (adherent or suspension) samples:

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

② Wash cells with normal saline (0.9% NaCl).

③ Homogenize 1×10^6 cells in 200 μL normal saline (0.9% NaCl) with a ultrasonic cell disruptor at 4℃.

④ Centrifuge at 10000 × g for 10 min at 4℃ 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).

② **Dilution of sample**

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

Sample type	Dilution factor
Human serum	50-100
Rat serum	50-100
10% Mouse kidney tissue homogenate	100-200
10% Mouse liver tissue homogenate	100-200
10% Mouse lung tissue homogenate	100-200
10% Mouse heart tissue homogenate	100-200
1×10^6 HL-60 cells	1
1×10^6 Jurkat cells	1

Note: The diluent is normal saline (0.9% NaCl). For the dilution of other sample types, please do pretest to confirm the dilution factor.

Operating steps

① Standard well: add 10 μL of standard with different concentrations into the well.
Sample well: add 10 μL of sample into the well.

② Add 140 μL of reaction working solution into each well.

③ Mix fully with fluorescence microplate for 5 s. Measure the fluorescence intensity at the excitation wavelength of 507 nm and the emission wavelength of 547 nm, as F_1.

④ Incubate at 37°C for 20 min protected from light. Measure the fluorescence intensity at the excitation wavelength of 507 nm and the emission wavelength of 547 nm, as F_2. (The standard curve is fitted to the standard well in F_1)

Calculation

The standard curve:

1. Average the duplicate reading for each standard.

2. Subtract the mean F_1 value of the blank (Standard #①) from all standard readings. This is the absoluted F_1 value.

3. Plot the standard curve by using absoluted F_1 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) samples:

Definition: The amount of enzyme in 1 L serum or plasma per 1 min that produce 1 μmol product at 37 °C is defined as 1 unit.

$$\text{LOX activity (U/L)} = (\Delta F - b) \div a \times f \div T$$

2. Tissue and cell samples:

Definition: The amount of enzyme in 1 g tissue or cell protein per 1 min that produce 1 μmol product at 37 °C is defined as 1 unit.

$$\text{LOX activity (U/gprot)} = (\Delta F - b) \div a \div C_{pr} \times f \div T$$

[Note]

ΔF: $\Delta F = \Delta F_{sample} - \Delta F_{blank}$, ($\Delta F_{sample} = F_2 - F_1$, $\Delta F_{blank} = F_2 - F_1$)

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

f: Dilution factor of sample before tested.

T: Reaction time, 20 min.

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Appendix I Performance Characteristics

1. Parameter:

Intra-assay Precision

Three rat serum 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)	0.09	0.20	0.40
%CV	2.2	4.9	3.2

Inter-assay Precision

Three rat serum were assayed 20 times in duplicate by three operators to determine precision between assays.

Parameters	Sample 1	Sample 2	Sample 3
Mean (U/L)	0.09	0.20	0.40
%CV	6.4	9.4	8.6

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 101%.

	Sample 1	Sample 2	Sample 3
Expected Conc(U/L)	0.09	0.20	0.40
Observed Conc(U/L)	0.089	0.204	0.404
Recovery rate (%)	99	102	101

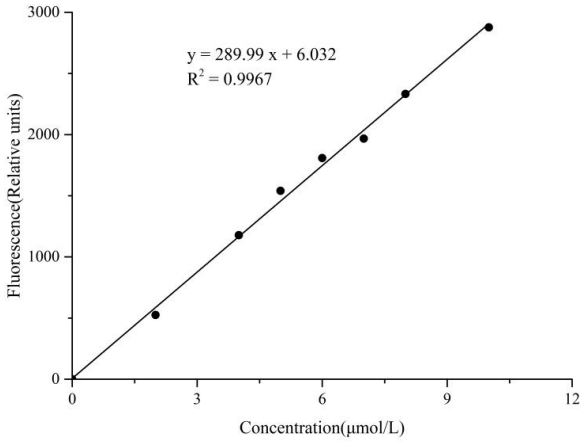
Sensitivity

The analytical sensitivity of the assay is 0.05 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 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	2	4	5	6	7	8	10
F_1 value	2082	2345	2980	3325	3657	3877	4219	4819
	1892	2680	3350	3729	3933	4031	4420	4910
Average F_1 value	1987	2513	3165	3527	3795	3954	4320	4865
Absoluted F_1 value	0	525	1178	1540	1808	1967	2332	2877



Appendix Π Example Analysis

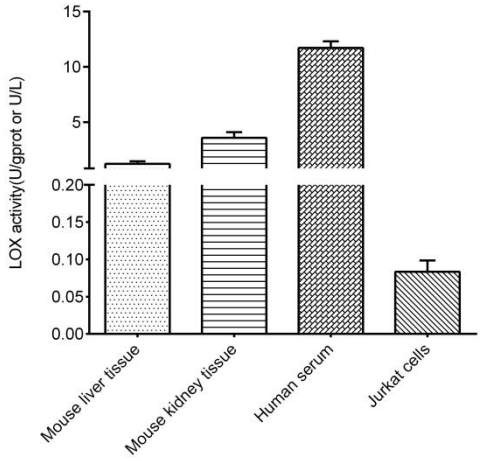
Example analysis：

Take 10 μL of 10% mouse liver tissue homogenate which dilute for 100 times and carry the assay according to the operation steps. The results are as follows:

Standard curve: y = 289.99 x + 6.032, the average F_1 value of the sample well is 2296, the average F_2 value of the sample well is 3694, the average F_1 value of the blank well is 1987, the average F_2 value of the blank well is 2110, $\Delta F = \Delta F_{sample} - \Delta F_{blank}$ = (3694 – 2296) – (2110 – 1987) = 1275, the concentration of protein is 19.70 gprot/L, and the calculation result is:

LOX activity (U/gprot) = (1275 – 6.032) ÷ 289.99 ÷ 19.70 × 100 ÷ 20 = 1.11 U/gprot

Detect 10% mouse liver tissue homogenate (the concentration of protein is 19.70 gprot/L, dilute for 100 times), 10% mouse kidney tissue homogenate (the concentration of protein is 16.17 gprot/L, dilute for 200 times), human serum (dilute for 50 times) and 1×10^6 Jurkat cells (the concentration of protein is 0.82 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.