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

Catalog No：E-BC-K861-M

Specification：48T(46 samples)/96T(94 samples)

Measuring instrument：Microplate reader（270-290 nm）

Detection range：5-339.5 U/L

Elabscience®Lipoxygenase (LOX) Activity 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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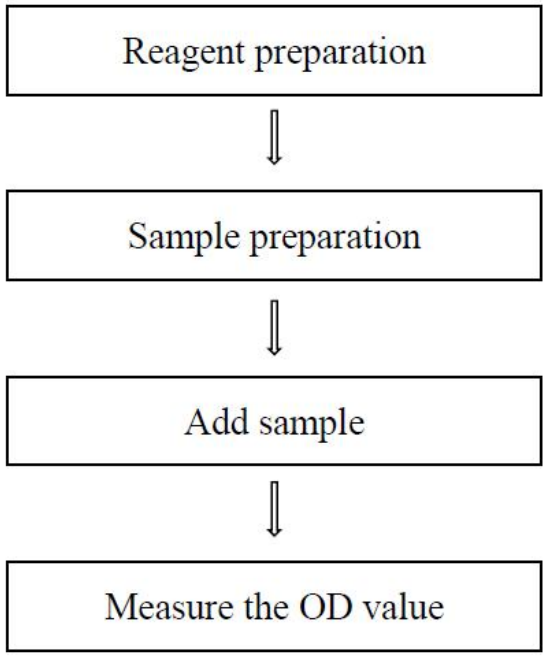
Assay summary



Intended use

This kit can measure lipoxygenase（LOX）activity in plant samples.

Detection principle

Lipoxygenase（LOX）is widely found in plant tissues, especially in seeds with high oil content such as soybean. LOX catalyzes the oxidation of unsaturated fatty acids, leading to membrane lipid peroxidation. It plays an important role in plant growth and development, maturation and senescence and stress.

LOX can catalyze linoleic acid oxidation, the oxidation product has the specific absorption peak at 280 nm. The activity of LOX can be calculated by measuring the change of absorbance value at 280 nm.

Kit components & storage

Item	Component	Size 1(48 T)	Size 2(96 T)	Storage
Reagent 1	Extraction Solution	50 mL × 1 vial	50 mL × 2 vials	2-8℃, 12 months
Reagent 2	Buffer Solution	15 mL × 1 vial	30 mL × 1 vial	2-8℃, 12 months
Reagent 3	Substrate	0.1 mL × 1 vial	0.1 mL × 1 vial	2-8℃, 12 months, shading light
	UV 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:

Microplate reader (270-290 nm, optimum wavelength: 280 nm), Incubator (37°C)

Reagents:

Double distilled water

Reagent preparation

① Equilibrate all reagents to room temperature before use.

② Preparation of substrate working solution：

Before testing, please prepare sufficient substrate working solution according to the test wells. For example, prepare 1209 μL of substrate working solution (mix well 9 μL of substrate and 1200 μL of buffer solution). Store at 2-8°C for 3 days protected from light.

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 minutes to remove insoluble material. Collect supernatant and keep it on ice for detection.

⑤ Meanwhile, determine the protein concentration of supernatant (E-BC-K168-M).

② Dilution of sample

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

Sample type	Dilution factor
10% Long bean	1
10% Green soy bean	2-5

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

① Avoid bubbles during the experiment.

② It is recommended to use fresh samples of plant seed.

Operating steps

① Blank well: Add 20 μL of double distilled water into the blank well.
Sample well: Add 20 μL of sample into the sample well.

② Add 160 μL of buffer solution into each well.

③ Add 20 μL of substrate working solution into each well.

④ Mix fully, measure the OD value of each well at 30 s and 1 min 30 s respectively at 280 nm with microplate reader, recorded as A_1, A_2, $\triangle A = A_2 - A_1$.

Calculation

The sample:

Tissue sample:

Definition: The amount of LOX in 1 g tissue protein per minute that catalyze the substrate resulting in a change of 0.01 units in the absorbance value

$$\underset{\text{(U/gprot)}}{\text{LOX activity}} = \Delta A \times V_{total} \div (C_{pr} \times V_{sample}) \div 0.01* \div T \times f$$

[Note]

ΔA: $A_2 - A_1$.

V_{total}: The total volume of the system, 0.2 mL.

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

V_{sample}: The volume of the sample, 0.02 mL.

0.01*: Definition of unit.

T: The time of reaction, 1 min.

f: Dilution factor of sample before test.

Appendix I Performance Characteristics

1. Parameter:

Inter-assay Precision

Three long bean 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)	8.40	68.50	188.00
%CV	4.3	4.1	3.6

Intra-assay Precision

Three long bean 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)	8.40	68.50	188.00
%CV	4.8	5.2	5.0

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%.

	Sample 1	Sample 2	Sample 3
Expected Conc. （U/L）	87.5	169	284
Observed Conc. （U/L）	86.6	163.9	286.8
recovery rate(%)	99	97	101

Sensitivity

The analytical sensitivity of the assay is 5 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.

Appendix II Example Analysis

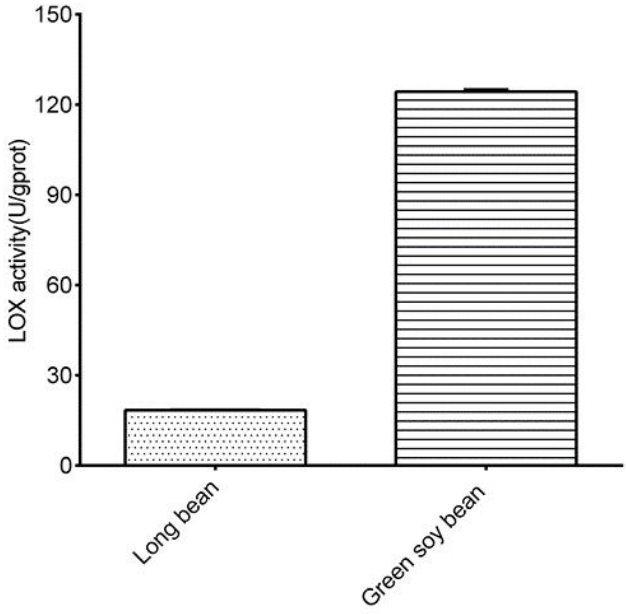
Example analysis：

Dilute 10% green soy bean for 2 times, take 20 uL of the diluted sample and carry the assay according to the operation steps. The results are as follows:

The average OD value of the sample (A_1) is 1.100, the average OD value of the sample (A_2) is 1.510, the concentration of protein in sample is 13.18 gprot/L, the calculation result is:

LOX activity (U/gprot) = (1.510 - 1.100) × 0.2 ÷ (13.18 ÷ 2) ÷ 0.02 ÷ 0.01 ÷ 1 × 2 =124.43 U/gprot

Detect 10% green soy bean (the concentration of protein is 13.18 gprot/L, dilute for 2 times), 10% long bean (the concentration of protein is 7.96 gprot/L) according to the protocol, the result is as follow：



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.