

PRODUCT INFORMATION & MANUAL

Lipoxygenase Activity Assay Kit (Colorimetric) NBP3-25863

For research use only.

Not for diagnostic or therapeutic procedures.

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Lipoxygenase Activity Assay Kit (Colorimetric)

Catalog No: NBP3-25863

Method: Colorimetric method

Specification: 96T (Can detect 94 samples without duplication)

Instrument: Microplate reader

Sensitivity: 5 U/L

Detection range: 5-339.5 U/L

Average intra-assay CV (%): 4.0

Average inter-assay CV (%): 5.0

Average recovery rate (%): 99

- ▲ This kit is for research use only.
- ▲ Instructions should be followed strictly, changes of operation may result in unreliable results.
- ▲ Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

General information

▲ 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	Specification	Storage
Reagent 1	Extraction Solution	50 mL × 2 vials	2-8°C , 12 months
Reagent 2	Buffer Solution	30 mL × 1 vial	2-8℃ , 12 months
Reagent 3	Substrate	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.

▲ Materials prepared by users



Instruments

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



Double distilled water, Tween-20

▲ Safety data

Some of the reagents in the kit contain dangerous substances. It should be avoided to touch the skin and clothing. Wash immediately with plenty of water if touching it carelessly. All the samples and waste material should be treated according to the relevant rules of laboratory's biosafety.

A Precautions

Before the experiment, please read the instructions carefully, and wear gloves and work clothes.

▲ The key points of the assay

Avoid bubbles during the experiment.

Pre-assay preparation

▲ Reagent preparation

- 1. Bring all reagents to room temperature before use.
- 2. Preparation of reagent 3 working solution:

Take 9 μ L of reagent 3, 30 μ L of Tween-20 and 1.161mL of reagent 2, mix fully for use. The prepared solution can be stored at 2-8°C with shading light for 3 days.

▲ Sample preparation

Weigh 0.020-1 g fresh plant tissue and wash with double distilled water, absorb moisture on the surface of tissue with filter paper. Then add PBS (0.01 M, pH 7.4) according to the ratio of the volume of reagent 1 (mL): the weight of the tissue (g) =9:1. Homogenize the sample on ice and centrifuge at 10000 g for 10 min at 4°C. Take the supernatant and preserve it on ice for detection. Meanwhile, determine the protein concentration of supernatant.

▲ Dilution of sample

It is recommended to take 2~3 samples with expected large difference to do pre-experiment before formal experiment and dilute the sample according to the result of the pre-experiment and the detection range (5-339.5 U/L).

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 reagent 1.

Assay protocol

▲ Plate set up

	1	2	3	4	5	6	7	8	9	10	11	12
Α	Α	Α	S15	S23	S31	S39	S47	S55	S63	S71	S79	S87
В	S1	S8	S16	S24	S32	S40	S48	S56	S64	S72	S80	S88
С	S2	S9	S17	S25	S33	S41	S49	S57	S65	S73	S81	S89
D	S3	S10	S18	S26	S34	S42	S50	S58	S66	S74	S82	S90
E	S4	S11	S19	S27	S35	S43	S51	S59	S67	S75	S83	S91
F	S5	S12	S20	S28	S36	S44	S52	S60	S68	S76	S84	S92
G	S6	S13	S21	S29	S37	S45	S53	S61	S69	S77	S85	S93
Н	S7	S14	S22	S30	S38	S46	S54	S62	S70	S78	S86	S94

Note: A, blank wells; S1-S94, sample wells.

▲ Detailed operation 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 reagent 2 into each well.
- (3) Add 20 μ L of reagent 3 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 , $\Delta A = A_2$ - A_1 .

▲ Summary operation table

	Blank well	Sample well
Double distilled water (µL)	20	
Sample (µL)		20
Regent 2 (µL)	160	160
Reagent 3 working solution (µL)	20	20

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 , $\Delta A = A_2$ - A_1 .

▲ Calculation

For plant 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.

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

Note:

 $\Delta A = A_2 - A_1$.

 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 Data

▲ 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 table. 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)

= $(1.510 - 1.100) \times 0.2 \div (13.18 \div 2) \div 0.02 \div 0.01 \div 1 \times 2 = 124.43 \text{ U/gprot}$