

## Plant Lipoxygenase (LOX) Activity Assay Kit

**Note:** Take two or three different samples for prediction before test.

**Operation Equipment:** Spectrophotometer

**Catalog Number:** AK0297

**Size:** 50T/48S

### Product composition:

Extract solution: Liquid 50 mL×1. Storage at 4°C . This reagent contains insoluble substances, just mix well before use.

Reagent I: Liquid 45 mL×1. Storage at 4°C .

Reagent II: Powder×1. Storage at 4°C . Add 5 mL of Reagent I to dissolve the powder, and add 0.1 mL of 0.2 mol/L NaOH for the solution to clear.

### Product Description

Lipoxygenase (LOX) is widely found in plant tissues, especially soybean seeds. LOX catalyzes the oxidation of unsaturated fatty acids, resulting in membrane lipid peroxidation. It plays an important role in plant growth and development, maturation and aging.

LOX catalyzes the oxidation of linoleic acid, the oxidation product has a characteristic absorption peak at 234 nm. The rate of increase in absorbance at 234 nm is measured to calculate the LOX activity.

### Reagents and Equipment Required but Not Provided.

Spectrophotometer, 1 mL quartz cuvette, refrigerated centrifuge, adjustable pipette, mortar/ homogenizer, ice and distilled water

### Procedure:

#### I. Sample Extraction:

Tissue sample: Weigh about 0.1 g of sample and add 1 mL of Extract solution, fully grind on ice, centrifuge at 16000 ×g and 4 °C for 20 minutes, and take the supernatant for testing.

#### II. Determination procedure:

1. Preheat the spectrophotometer for more than 30 minutes, adjust the wavelength to 234 nm, and set zero with distilled water.
2. Reagent I is incubated in a water bath at 25°C for more than 30 minutes.
3. Blank tube: In a 1 mL quartz cuvette, add 100 μL of distilled water, 800 μL of Reagent I and 100 μL of Reagent II, after mix them quickly, measure at 234 nm, record the absorbance at 15s and 75s, and record them as A1 and A2.
4. Test tube: Add 100 μL of supernatant, 800μL of Reagent I and 100μL of Reagent II to a 1 mL quartz cuvette, after mix them quickly, measure at 234 nm, record the absorbance at 15s and 75s, and record them as A3 and A4.

### III. Calculations

#### 1. Protein concentration:

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the absorbance of 0.001 change at 37 °C in 1 milliliter reaction system per minute every milligram protein.

$$\text{LOX activity (U/mg)} = [(A4-A3)-(A2-A1)] \div 0.001 \div (C_{pr} \times V_s) \div T \times V_r = 10^4 \times [(A4-A3)-(A2-A1)] \div C_{pr}$$

#### 2. Sample weight:

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the absorbance of 0.001 change at 37 °C in 1 milliliter reaction system per minute every gram tissue sample.

$$\text{LOX activity (U/g)} = [(A4-A3)-(A2-A1)] \div 0.001 \div (W \times V_s \div V_e) \div T \times V_r = 10^4 \times [(A4-A3)-(A2-A1)] \div W$$

C<sub>pr</sub>: Supernatant protein concentration, mg/mL;

T: Reaction time, 1 minute;

V<sub>s</sub>: Sample volume, 0.1 mL;

V<sub>e</sub>: Extraction volume, 1 mL;

V<sub>r</sub>: Reaction volume, 1 mL;

W: Sample weight, g.

#### Notes:

1. Reagent II is susceptible to spontaneous oxidation, resulting in a high blank tube measurement value, which must be prepared before use.
2. Sample preparing process and other processes need to be performed on ice, and the enzyme activity measurement must be completed on the same day.
3. Before the formal experiment, do 1-2 pre-experiments to ensure that ΔA is in the range of 0.02 - 1.2. If the reaction is a obvious suspension, please measure it after dilution.

#### References:

[1] Dou S, Liu S, Xu X, et al. Octanal inhibits spore germination of *Penicillium digitatum* involving membrane peroxidation[J]. *Protoplasma*, 2017, 254(4): 1539- 1545.

#### Related products:

AK0536/AK0535 Free fatty acid (FFA) content detection kit

AK0384/AK0383 Lipase (LPS) activity detection kit

AK0262/AK0261 Ethanol dehydrogenase (ADH) activity detection kit

AK0492/AK0491 Pyruvate decarboxylase (PDC) activity detection kit

AK0530/AK0529 Triglyceride (TG) content detection kit

AK0412/AK0411 Free cholesterol (FC) content detection kit

AK0269/AK0268 Acetaldehyde dehydrogenase (ALDH) activity detection kit

AK0323/AK0322 Acetyl-coa carboxylase (ACC) activity detection kit

AK0327/AK0326 Total cholesterol (TC) assay kit