Plant Lipoxygenase (LOX) Activity Assay Kit

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

Operation Equipment: Spectrophotometer/ microplate reader

Catalog Number: AK0296

Size:100T/96S

Product composition:

Extract solution: Liquid 110 mL×1. Storage at 2-8℃.

Powder I: Powder×1. Storage at 2-8℃. Pour the Powder I into the Extraction Solution before use. The solution is a suspension. Shake it well before use.

Reagent I: Liquid 20 mL×1. Storage at 2-8℃.

Reagent II: Liquid 3 mL×1. Storage at 2-8℃.

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/microplate reader, micro quartz cuvette/ 96-well flat-bottom plate (UV), 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℃ for 20 minutes, and take the supernatant for test.

II. Determination procedure:

1. Preheat the spectrophotometer/ microplate reader 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℃ for more than 30 minutes.
3. Blank tube: In a micro quartz cuvette/ 96-well flat-bottom plate (UV), add 20 μL of distilled water, 160 μL of Reagent I and 20 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. The blank tube only needs to be done 1-2 times.
4. Test tube: Add 20 μL of supernatant, 160 μL of Reagent I and 20 μL of Reagent II to a micro quartz cuvette/96-well flat-bottom plate (UV), after mix them quickly, measure at 234 nm, record the

absorbance at 15s and 75s, and record them as A3 and A4.

III. Calculations

a. The calculation formula of micro quartz cuvette is as follows

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℃ in 1 milliliter reaction system per minute every milligram protein.

LOX activity (U/mg) = [(A4-A3)-(A2-A1)]÷0.001÷(Cpr×Vs)÷T×Vr = 10^4×[(A4-A3)-(A2-A1)]÷Cpr

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℃ in 1 milliliter reaction system per minute every gram tissue sample.

LOX activity (U/g) = [(A4-A3)-(A2-A1)]÷0.001÷(W×Vs÷Ve)÷T×Vr = 10^4×[(A4-A3)-(A2-A1)]÷W

Cpr: Supernatant protein concentration, mg/mL;

T: Reaction time, 1 minute;

Vs: Sample volume, 0.02 mL;

Ve: Extraction volume, 1 mL;

Vr: Reaction volume, 0.2 mL

W: Sample weight, g.

b. The calculation formula of 96 well plate is as follows

1. Protein concentration:

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the absorbance of 0.0006 change at 37℃ in 1 milliliter reaction system per minute every milligram protein.

LOX activity (U/mg prot) = [(A4-A3)-(A2-A1)]÷0.0006÷(Cpr×Vs)÷T× Vr

= 16667×[(A4-A3)-(A2-A1)]÷Cpr

2. Sample weight:

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the absorbance of 0.0006 change at 37 ℃ in 1 milliliter reaction system per minute every gram tissue sample.

LOX activity (U/g weigh) = [(A4-A3)-(A2-A1)]÷0.0006÷(W×Vs÷Ve)÷T× Vr

= 16667×[(A4-A3)-(A2-A1)]÷W

Cpr: Supernatant protein concentration, mg/mL;

T: Reaction time, 1 minute;

Vs: Sample volume, 0.02 mL;

Ve: Extraction volume, 1 mL;

Vr: Reaction volume, 0.2 mL

W: Sample weight, g.

Notes:

1. Sample preparing process need to be performed on ice, and the enzyme activity measurement must be completed on the same day.
2. 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.
3. For some samples whose color is too deep to affect the result determination, 3-5mg activated carbon can be added to shake and absorb the pigment during sample extraction, centrifuge the supernatant for use.

References:

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.