Plant Lipoxygenase (LOX) Activity Assay Kit

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

Operation Equipment: Spectrophotometer

Catalog Number: BC0320

Size: 50T/48S

Product Composition: Before use, please carefully check whether the volume of the reagent is consistent with the volume in the bottle. If you have any questions, please contact Solarbio staff in time.

Reagent name	Size	Preservation Condition
Extract solution	Liquid 60 mL×1	2-8℃
Powder I	Powder×1	2-8℃
Reagent I	Liquid 55 mL×1	2-8℃
Reagent II	Liquid 6 mL×1	2-8℃

Solution Preparation:

1. Extract solution: Pour the Powder I into the Extraction Solution before use. The solution is a suspension. Shake it well before use.

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℃ 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℃ 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. The blank tube only needs to be done 1-2 times.

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

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

$=10^4\times[(A4-A3)-(A2-A1)]\div Cpr$

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

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

$=10^4\times[(A4-A3)-(A2-A1)]\div W$

Cpr: Supernatant protein concentration, mg/mL;

T: Reaction time, 1 minute;

Vs: Sample volume, 0.1 mL;

Ve: Extraction volume, 1 mL;

Vr: Reaction volume, 1 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.

Related products:

BC0590/BC0595 Free fatty acid (FFA) content detection kit

BC2340/BC2345 Lipase (LPS) activity detection kit

BC1080/BC1085 Ethanol dehydrogenase (ADH) activity detection kit

BC1070/BC1075 Pyruvate decarboxylase (PDC) activity detection kit

BC0620/BC0625 Triglyceride (TG) content detection kit

BC1890/BC1895 Free cholesterol (FC) content detection kit