Indoleacetic Acid Oxidase Activity Assay Kit

Note: The reagents are subject to change, please note and follow these instructions closely.

Operation Equipment: Spectrophotometer/ Microplate Reader

Catalog Number: BC4105

Size: 100T/48S

Components:

Extract solution: Liquid 60ml×1, store at 2-8℃.

Reagent I: Powder×1, store at 2-8℃, dissolve with 5ml of distilled water before use. It could be stored at 2-8℃ for two weeks.

Reagent II: Liquid 3ml×1, store at 2-8℃.

Reagent III: Powder×1, store at -20℃, dissolve with 5.71ml of 50% alcohol (alcohol volume: water volume=1:1) before use. It can be stored at -20℃ for two weeks after dispensing to avoid repeated freezing and thawing.

Reagent IV: Liquid 20ml×1, store at 2-8℃.

Reagent V: Powder×1, store at 2-8℃, dissolve with 10ml of reagent IV for use. It could be stored at 2-8℃ for two weeks.

Standard: Powder×1, 10 mg of indoleacetic acid, store at -20℃ and avoid light. Add 1.14ml of 50% alcohol (alcohol volume: water volume=1:1) for use to make 50umol/mL standard solution. It can be stored at -20℃ for two weeks after dispensing to avoid repeated freezing and thawing.

Product Description:

Indoleacetic acid (IAA) is deactivated and damaged under the catalyzation of indoleacetic acid oxidase. IAA oxidase can regulate the level of indoleacetic acid in plants and affect plant growth.

In the condition of inorganic acid, IAA react with $FeCl_3$ to form red product, which has absorption peak at 530nm.The enzyme activity can be expressed by the rate of destruction of IAA.

Reagents and Equipment Required but Not Provided:

Spectrophotometer/ microplate reader, micro quartz cuvette/96 well flat-bottom plate, water bath, low temperature centrifuge, adjustable transferpettor, mortar/homogenizer/cell ultrasonicator , alcohol, ice and distilled water.

Sample preparation:

1. Tissue: Add 1 ml of extract solution into 0.1g of tissue, fully grinding on ice. centrifuge at 12000g and 4℃ for 15 min, supernatant on ice is used for test.
2. Cells or microbial sample: collect cells or microbial sample to centrifuge and remove supernatant. Suggested 5 million with 1mL of extract solution, split bacteria and cell with ultrasonication (power 200W, work time 3s, interval 10s, for 30 times). Centrifuge at 12000g and 4℃ for 15min, supernatant

on ice is used for test.

Procedure:

1. Preheat spectrophotometer/ microplate reader for 30min, adjust the wavelength to 530 nm, set spectrophotometer counter to zero with distilled water.
2. Dilution of standard solutions : dilute standard solution with distilled water to 0.4, 0.2, 0.1, 0.05, 0.025, 0.0125μmol/mL for use.
3. Add the following reagents:

Reagent(μL)	Test tube (A3)	Contrast tube (A4)	Standard tube (A1)	Blank tube (A2)
Extract solution	40	40	-	-
Reagent I	8	8	-	-
Reagent II	8	8	-	-
Reagent III	16	16	-	-
Sample	8	-	-	-
Mix thoroughly, 30℃ water bath for 30 min			-	-
Reagent IV	80	80	80	80
Sample	-	8	-	-
Standard	-	-	80	-
ddH_2O	-	-	-	80
Centrifuge 10000 g at room temperature for 10 min, take the supernatant to be measured (Standard tube and Blank tube without centrifugation directly take 750 μL for the following experiment)				
supernatant	130	130	-	-
Standard mixture	-	-	130	-
Blank mixture	-	-		130
Reagent V	70	70	70	70
Store at 30℃ and avoid light for 30 min, detect at 530 nm, record as A1, A2, A3, A4, calculate ΔA_S= A1-A2, ΔA_T =A4-A3. Each test tube should be provided with one contrast tube. Standard curve and blank tube only need to be measured once or twice.				

Calculation:

1 Make standard curve:

Taking the concentration of each standard solution as the x-axis and its corresponding ΔA_S as the y-axis, draw a standard curve to get the standard equation y = kx + b, and bring ΔA_T into the equation to get x (μmol/mL).

2 Sample weight:

Unit definition: One unit of enzyme activity is defined as the amount of enzyme that catalyzes the consumption of 1 nmol IAA per min every gram tissue weight.

IAA oxidase (umol/g FW) = x ×V× 1000÷ (W÷Ve×Vs)÷T=333×x ÷ W

3 Protein concentration:

Unit definition: One unit of enzyme activity is defined as the amount of enzyme that catalyzes the consumption of 1 nmol IAA per min every. mg tissue protein

IAA oxidase (umol/mg prot) = x× V× 1000÷ (Vs × Cpr)÷T=333× x÷Cpr

4 Cells or bacteria:

Unit definition: One unit of enzyme activity is defined as the amount of enzyme that catalyzes the consumption of 1 nmol IAA per min every 10^4 cells.

IAA oxidase (U/10^4 cell) = x× V× 1000÷ (N÷Ve×Vs)÷T=333× x÷N

V: total react volume, 0.08mL;

1000:1μmol=1nmol

Cpr: Sample concentration (mg/mL);

W: Sample weight(g);

Vs: Sample volume (mL), 0.008 mL;

Ve: Extraction solution volume(mL), 1mL

T: Reaction time (min), 30 min

N: Number of cells, in the tens of thousands

Note:

1. When ΔA is greater than 0.4 or A4 is greater than 1, it is recommended that the sample be diluted with the extract and assayed; when ΔA is too small, increase the enzymatic reaction time (1h or 2h) or increase the volume of sample added to the assay.
2. Reagent I cannot use when turning to blank. Take protective measures because reagent II is toxic.

Experimental Examples:

1. Take 0.1g of red beans and add 1mL extract to homogenize and grind, take the supernatant and operate according to the measurement steps, measure by the 96 well plate and calculate ΔA=A2-A1=0.477-0.402=0.075, bring standard curve line y=2.7049x-0.0154, x=0.0334, calculate the enzyme based on the sample weight:

IAA Activity（U/g weight）=333×ΔA÷W=333×0.0334÷0.1=111.22 U/g weight。

Related Products:

BC4070/BC4075 Tannase Activity Assay Kit

BC4080/BC4085 Cinnamic acid 4-hydroxylase(C4H) Activity Assay Kit

BC4090/BC4095 Anthocyanidin Reductase Activity Assay Kit