Indoleacetic Acid Oxidase Activity Assay Kit

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

Operation Equipment: Spectrophotometer

Catalog Number: NA0349

Size: 50T/24S

Components:

Extract solution: 40ml×1 bottle, storage at 4℃.

Reagent I: powder×1 bottle, storage at 4℃, dissolve with 5ml of distilled water before use;

Reagent II: powder×1 bottle, storage at 4℃, dissolve with 3ml of distilled water before use;

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

Reagent IV: 60ml×1 bottle, storage at 4℃.

Reagent V: powder×1 bottle, storage at 4℃, dissolve with 30ml of reagent IV for use.

Standard: powder×1 bottle, 10 mg indoleacetic acid, storage 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℃ 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, low temperature centrifuge, adjustable transferpettor, water bath, 1ml quartz cuvette, mortar, 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 12000rpm and 4℃ for 15min, 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 20%, work time 3s, interval 10s, for 30 times). Centrifuge at 12000rpm and 4℃ for 15min, supernatant on ice is used for test.

Procedure:

1. Preheat spectrophotometer for 30min, adjust the wavelength to 530 nm, set the counter to zero with

distilled water.

2. Dilute standard solution with distilled water to 0.2umol/mL, 0.1umol/mL, 0.05umol/mL, 0.025umol/mL, 0.0125umol/mL for use.
3. Add the following reagents:

Reagent name(ul)	Test tube (A3)	Contrast tube (A4)	Standard tube (A1)	Blank tube (A2)
Extract solution	200	200	-	-
Reagent I	40	40	-	-
Reagent II	40	40	-	-
Reagent III	80	80	-	-
Sample	40	-	-	-
Mix thoroughly, 30℃ water bath for 30 min			-	-
Reagent IV	400	400	400	400
Sample	-	40	-	-
Standard	-	-	400	-
ddH_2O	-	-	-	400
centrifuge at 10000g for 10min, get the supernatant			-	-
supernatant	750	750	-	-
Standard mixture			750	750
Reagent V	400	400	400	400
Store at 30℃ and avoiding light for 30min, detect at 530nm, A1, A2, A3, A4, calculate ΔA(standard)= ΔA(S)= A1-A2, ΔA(test)=ΔA(T) =A4-A3.				

Calculation:

1 Make standard curve:

standard liquid as the X-axis, ΔA(S) as Y-axis ordinate, establish the standard curve and get formula y=kx+b. Take ΔA(T) to formula, get x(umol/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×ΔA ÷ 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÷ (500÷Ve×Vs)÷T=0.667×ΔA

V: total react volume, 0.4mL; 1000:1μmol=1nmol

Cpr: Sample concentration (mg/mL);

W: Sample weight(g);

Vs: Sample volume (mL), 0.04 mL;

Ve: Extraction solution volume(mL), 1mL

T: Reaction time (min), 30 min

Note:

1. Dilute sample with extract solution if △A>0.5 or A4>1; Increase react time or sample volume if △A is too low.
2. Reagent 1 cannot use when turning to blank. Take protective measures because reagent 2 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, and calculate ΔA=A4-A3=0.800-0.668=0.132, bring standard curve line y=4.8418 x-0.042, x=0.0359, calculate the enzyme based on the sample weight:

IAA Activity（U/g weight）=333×ΔA÷W=333×0.0359÷0.1=119.55 U/g weight。

Related Products:

NA0359/NA0358 Tannase Activity Assay Kit

NA0357/NA0356 Cinnamic acid 4-hydroxylase(C4H) Activity Assay Kit

NA0351/NA0350 Anthocyanidin Reductase Activity Assay Kit