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Indoleacetic Acid Oxidase Activity Assay Kit

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

Operation Equipment: Spectrophotometer/ Microplate Reader

Catalog Number: AK0349

Size: 100T/48S

Components:

Extract solution: 60ml×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: 30ml×1 bottle, storage at 4℃ .

Reagent V: powder×1 bottle, storage at 4℃, dissolve with 15ml of reagent IV for use;

Standard: powder×1 bottle, 10 mg of indoleacetic acid, storage at -20C 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/ microplate reader, micro quartz cuvette/96 well flat-bottom UV plate, water bath, low temperature centrifuge, adjustable transferpettor, 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 4C 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 20%, work time 3s, interval 10s, for 30 times). Centrifuge at 12000rpm and 4C for 15min, supernatant on ice is used for test.

Procedure:

1. Preheat spectrophotometer/ microplate reader for 30min, adjust the wavelength to 530 nm, set the

counter to zero with distilled water.

2. Dilute standard solution with distilled water to 0.4umol/mL,0.3umol/mL, 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	40	40	-	-
Reagent I	8	8	-	-
Reagent II	8	8	-	-
Reagent III	16	16	-	-
Sample	8	-	-	-
Mix thoroughly, 30℃ water bath for 30 min			-	-
ReagentIV	80	80	80	80
Sample	-	8	-	-
Standard	-	-	80	-
ddH_2O	-	-	-	80
10000g centrifuge for 10min, get supernatant			-	-
supernatant	130	130	-	-
Standard mixture			130	130
Reagent V	70	70	70	70
Storage at 30 ℃ and avoid light for 30 min, detect at 530 nm, 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 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, 0.08mL; 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

Note:

1. Dilute sample with extract solution if ΔA>0.4 or A4>1, then determination of absorbance; increase react time (1 h or 2 h) and sample volume if ΔA is too low, then determination of absorbance.
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, 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:

AK0356/AK0355 Tannase Activity Assay Kit

AK0354/AK0353 Cinnamic acid 4-hydroxylase(C4H) Activity Assay Kit

AK0352/AK0351 Anthocyanidin Reductase Activity Assay Kit