Ornithine Aminotransferase(δ-OAT) Activity Assay Kit

Product Code: 55750

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Method: Spectrophotometric method

Product Introduction

Proline is an important osmotic regulator that helps plants adapt to stress conditions. In higher plants, proline is synthesized through two pathways based on the initial substrate: the glutamate (Glu) pathway and the ornithine (Orn) pathway.

Ornithine aminotransferase (δ-OAT) is a key enzyme in the ornithine-based pathway for proline synthesis and plays an important role in plant stress adaptation. Ornithine and α-ketoglutarate undergo an aminotransferase reaction catalyzed by ornithine aminotransferase and NADH to generate pyrroline-5-carboxylic acid (P5C), while producing NAD. The change in absorbance at 340 nm reflects the level of ornithine aminotransferase activity.

Package Contents

Size	Code	Component	Quantity
50T	55750.1	Reagent One	1 bottle
50T	55750.2	Reagent Two	1 bottle
50T	55750.3	Reagent Three	1 bottle
50T	55750.4	Reagent Four	1 bottle
50T	55750.5	Extraction Solution	1 bottle
50T	55750.m	Instruction Manual	1 copy

Quality and Safety Information

Material or Package Name	Quality Standard	Main Toxicity
Reagent One	—	—
Reagent Two	—	—
Reagent Three	—	—
Reagent Four	—	—
Extraction Solution	-	--

Transportation and Storage

Transportation	Transport with ice packs.
Storage	Store Reagent IV at -20 °C. Store the other components at 2-8 °C protected from light. Shelf life: 180 days.

Instructions for Use

1. Enzyme Solution Extraction

Tissue Samples

Use a sample mass (g) to extraction solution volume (mL) ratio of 1:5-10. It is recommended to weigh approximately 0.1 g sample and

add 1 mL extraction solution.

1. Add the extraction solution to the sample.
2. Homogenize in an ice bath.
3. Centrifuge at 4 °C, 10000g for 10 min.
4. Collect the supernatant and keep it on ice for testing.

Cell Samples

Use a cell count (10^4cells) to extraction solution volume (mL) ratio of 500-1000:1. It is recommended to add 500×10^4cells to 1 mL extraction solution.

1. Disrupt the cells by ultrasonic treatment in an ice bath at 300 W, with 3 seconds sonication and 7 seconds interval, for a total time of 3 min.
2. Centrifuge at 4 °C, 10000g for 10 min.
3. Collect the supernatant and keep it on ice for testing.

Liquid Samples

Test liquid samples directly.

2. Reagent Preparation

- Before use, add 20 mL Reagent I to Reagent II and dissolve thoroughly. Store unused reagent at 4 °C.
- Before use, add 20 mL Reagent I to Reagent III and dissolve completely. Store unused reagent at 4 °C.
- Before use, add 10 mL Reagent I to each bottle of Reagent IV and dissolve completely. Prepare fresh and use immediately.

3. Measurement Procedure

1. Preheat the spectrophotometer for 30 min and set the wavelength to 340 nm.
2. Preheat the prepared Reagents II, III, and IV at 37 °C for 5 min.
3. Use a 1 mL quartz cuvette. Add 300 μL Reagent II, 300 μL Reagent III, 300 μL Reagent IV, and 100 μL crude enzyme solution in sequence.
4. Mix thoroughly.
5. Record the initial absorbance at 340 nm as A1.
6. React at 37 °C for 10 min and record the absorbance as A2.
7. Calculate ΔA = A1 - A2.

> Powdered reagents must be prepared by the user before use.

Activity Calculation

Calculated by Sample Protein Concentration

Unit definition: consumption of 1 nmol NADH per milligram of tissue protein per minute is defined as one unit of enzyme activity.

$$\delta\text{-OAT (nmol/min/mg prot)} = \Delta A \div (\varepsilon \times d) \times V_{\text{total reaction}} \div (V_{\text{sample}} \times Cpr) \div T = 160.77 \times \Delta A \div Cpr$$

Calculated by Sample Mass

Unit definition: consumption of 1 nmol NADH is defined as one enzyme activity unit.

$$\delta\text{-OAT (nmol/min/g fresh weight)} = \Delta A \div (\varepsilon \times d) \times V_{\text{total reaction}} \div (W \times V_{\text{sample}} \div V_{\text{total sample}}) \div T = 160.77 \times \Delta A \div W$$

Calculated by Cell Number

Unit definition: consumption of 1 nmol NADH per 10^4cells per minute is defined as one enzyme activity unit.

$$\delta\text{-OAT (nmol/min/}10^4\text{cells)} = \Delta A \div (\varepsilon \times d) \times V_{\text{total reaction}} \div (V_{\text{sample}} \times \text{cell number} \div V_{\text{total sample}}) \div T = 160.77 \times \Delta A \div \text{cell count}$$

Calculated by Liquid Volume

Unit definition: consumption of 1 nmol NADH per milliliter of liquid per minute is defined as one enzyme activity unit.

δ-OAT (nmol/min/mL) = $\Delta A \div (\varepsilon \times d) \times V_{total\ reaction} \div V_{sample} \div T = 160.77 \times \Delta A$

Formula Parameters

$V_{total\ reaction}$	Total volume of the reaction system, 1 mL
ε	NADH molar extinction coefficient, 6.22×10^3 L/mol/cm
d	Optical path length of the cuvette, 1 cm
V_{sample}	Volume of sample added, 0.1 mL
$V_{total\ sample}$	Volume of extract added, 1 mL
T	Reaction time, 10 min
Cpr	Sample protein concentration, mg/mL
W	Sample mass, g

Precautions

- Before the formal assay, select 2-3 samples with large expected differences for a preliminary test.
- Instruments and supplies to be prepared by the user: balance, refrigerated centrifuge, mortar, UV-visible spectrophotometer, and 1 mL quartz cuvette.