Ornithine Aminotransferase(δ-OAT) Activity Assay Kit

Product Code: 55794

Ornithine Aminotransferase Activity Assay Kit - Microplate Method

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Assay target: Ornithine Aminotransferase (δ-OAT) Activity

Product Introduction

Proline is an important osmotic regulator that helps plants adapt to stress conditions. In higher plants, proline metabolism is divided into two synthesis pathways according to the initial substrate: the glutamate (Glu) pathway and the ornithine (Orn) pathway.

Ornithine aminotransferase (δ-OAT) is a key enzyme in the pathway that synthesizes proline using ornithine as the precursor, and it plays an important role in plant adaptation to stress conditions.

Under the action of ornithine aminotransferase and NADH, ornithine and α-ketoglutaric acid undergo an aminotransferase reaction to form pyrroline-5-carboxylic acid (P5C), while producing NAD. The activity level of ornithine aminotransferase is reflected by measuring the change in absorbance at 340 nm.

Package Contents

Size	Code	Component	Quantity
100T	55794.1	Reagent I	1 bottle
100T	55794.2	Reagent II	1 bottle
100T	55794.3	Reagent III	1 bottle
100T	55794.4	Reagent IV	1 bottle
100T	55794.5	Extraction Solution	1 bottle
100T	55794.m	Instructions	1 copy

Quality Standards and Safety Information

Raw Material or Packaging Name	Quality Standard	Main Toxicity
Reagent I	--	--
Reagent II	--	--
Reagent III	--	--
Reagent IV	--	--
Extraction Solution	--	--

Transportation and Storage

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

Instructions for Use

1. Enzyme Solution Extraction

1.1 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 of sample and add 1 mL of Extraction Solution.

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

1.2 Cell Samples

Use a cell number (10^4cells) to extraction solution volume (mL) ratio of 500-1000:1. It is recommended to add 1 mL of Extraction Solution to 500 ten thousand cells.

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

1.3 Liquid Samples

Test liquid samples directly.

2. Reagent Preparation

1. Before use, add 8 mL of Reagent I to Reagent II and dissolve thoroughly. Store unused prepared reagent at 4°C.
2. Before use, add 8 mL of Reagent I to Reagent III and dissolve thoroughly. Store unused prepared reagent at 4°C.
3. Before use, add 4 mL of Reagent I to each bottle of Reagent IV and dissolve thoroughly. Prepare fresh and use immediately.

3. Assay Procedure

1. Preheat the microplate reader 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. In a 96-well plate, sequentially add 60 μL of Reagent II, 60 μL of Reagent III, 60 μL of Reagent IV, and 20 μL of crude enzyme solution.
4. Mix thoroughly.
5. Record the initial absorbance at 340 nm as A1.
6. React at 37°C for 10 min, then record the absorbance at 340 nm as A2.
7. Calculate ΔA = A1 - A2.

> Powdered reagents need to be prepared by the user.

Activity Calculation

1. Calculation by Sample Protein Concentration

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

$$\delta\text{-OAT (nmol/min/mg prot)} = \Delta A \div (\varepsilon \times d) \times V_{\text{total reaction}} \div (V_{\text{sample}} \times Cpr) \div T = 321.54 \times \Delta A \div Cpr$$

2. Calculation by Sample Mass

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

$$\delta\text{-OAT (nmol/min/g fresh weight)} = \Delta A \div (\varepsilon \times d) \times V_{\text{total reaction}} \div (W \times V_{\text{sample}} \div V_{\text{total sample}}) \div T = 321.54 \times \Delta A \div W$$

3. Calculation by Cell Number

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

δ-OAT (nmol/min/10^4cells) = ΔA ÷ (ε × d) × $V_{total\ reaction}$÷ (V_{sample}× number of cells ÷ $V_{total\ sample}$) ÷ T = 321.54 × ΔA ÷ number of cells

4. Calculation by Liquid Volume

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

δ-OAT (nmol/min/mL) = ΔA ÷ (ε × d) × $V_{total\ reaction}$÷ V_{sample}÷ T = 321.54 × ΔA

Formula Parameters

$V_{total\ reaction}$	Total volume of the reaction system, 0.2 mL
ε	NADH molar extinction coefficient, 6.22 × 10^3L/mol/cm
d	96-well plate optical path length, 0.5 cm
V_{sample}	Added sample volume, 0.02 mL
$V_{total\ sample}$	Added extraction solution volume, 1 mL
T	Reaction time, 10 min
Cpr	Sample protein concentration, mg/mL
W	Sample mass, g

Precautions

- Before formal measurement, select 2-3 samples with large expected differences for preliminary testing.
- Instruments and supplies required but not provided: balance, refrigerated centrifuge, mortar, microplate reader, and 96-well plate.