Ornithine Aminotransferase(δ-OAT) Activity Assay Kit

Product Code: 55187

Ornithine Aminotransferase (δ-OAT) Activity Assay Kit, Micro Method

Product Information

Product Code	55187
Assay Format	Micro method
Size	100T

Product Introduction

Proline is an important osmotic regulator in plants and supports adaptation to stress conditions. In higher plants, proline metabolism includes glutamate (Glu) and ornithine (Orn) synthesis pathways, classified by their initial substrates.

Ornithine aminotransferase (δ-OAT) is a key enzyme in the pathway that uses ornithine as a precursor for proline synthesis. Ornithine and α-ketoglutaric acid undergo an aminotransferase reaction catalyzed by δ-OAT in the presence of NADH, generating pyrroline-5-carboxylic acid (P5C) and NAD. The change in absorbance at 340 nm reflects δ-OAT activity.

Package Contents

Code	Component	Quantity
55187.1	Reagent One	1 bottle
55187.2	Reagent Two	1 bottle
55187.3	Reagent Three	1 bottle
55187.4	Reagent Four	1 bottle
55187.5	Extract Solution	1 bottle
55187.m	Instruction Manual	1 copy

Quality and Safety Information

Component	Quality Standard	Main Toxicity
Reagent One	--	--
Reagent Two	--	--
Reagent Three	--	--
Reagent Four	--	--
Extract Solution	--	--

Transportation and Storage

Condition	Requirement
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	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 tissue and add 1 mL 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. Keep the supernatant on ice for testing.

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 extraction solution to 500 x 10,000 cells.

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

Liquid Samples

Test liquid samples directly.

2. Reagent Preparation

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

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

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, add 60 μL Reagent II, 60 μL Reagent III, 60 μL Reagent IV, and 20 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, then record the absorbance as A2.
7. Calculate ΔA = A1 - A2.

Activity Calculation

Calculation by Sample Protein Concentration

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

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

Calculation by Sample Mass

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

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

Calculation by Cell Number

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

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

Calculation by Liquid Volume

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

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

Formula Parameters

Parameter	Definition	Value
$V_{reaction\ total}$	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}	Sample volume added	0.02 mL
$V_{sample\ total}$	Extraction solution volume 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 preliminary testing.
- Required instruments and supplies include a balance, refrigerated centrifuge, mortar, microplate reader, and 96-well plate.