Ornithine aminotransferase (δ-OAT) Activity Assay Kit

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

Detection equipment: Spectrophotometer

Cat No: NA0292

Size: 50T/48S

Components:

Extract solution : 55 mL×1, store at 4℃ and protect from light.

Reagent I: 70 mL×1, store at 4℃.

Reagent II: Powder×2, store at 4℃ and protect from light. Add 10 mL of Reagent I when the solution will be used. Unused reagent can store at 4℃ after packing.

Reagent III: Powder×2, store at 4℃ and protect from light. Add 10 mL of Reagent I when the solution will be used. Unused reagent can store at 4℃ after packing.

Reagent IV: Powder×2, store at -20℃ and protect from light. Add 10 mL of distilled water when the solution will be used. Unused reagent can store at -20℃ for one week after packing.

Description:

Ornithine aminotransferase (δ-OAT) is one of the key enzymes for the synthesis of proline by ornithine as a precursor, which plays an important role in adapting plants to stress. Ornithine and α-ketoglutarate can undergo acyl transfer reaction under the action of δ-OAT and NADH to produce NAD and pyrrolaldehyde-5-carboxylic acid (P5C). NADH has a special absorption peak at 340 nm. By measuring the change in absorbance at 340 nm, the level of δ-OAT activity can be calculated.

Required but not provided

Spectrophotometer, low temperature centrifuge, water-bath/constant temperature incubator, transferpettor, 1 mL quartz cuvette, homogenizer, ice, distilled water and EP tubes.

Protocol:

I. Crude enzyme extraction:

1. Tissue:

The mass of tissue (g): the volume of extract (mL) is 1:5~10(it is suggested to take about 0.1 g of tissue, add 1 mL of Extract solution), fully grinding on ice. Centrifuge at 12000 rpm for 10 minutes at 4℃, take the supernatant and place it on ice for test.

2. Bacteria or cells

Collecting bacteria or cells into the centrifuge tube. The supernatant is discarded after centrifugation. it is suggested to take about 5 million bacteria/cell and add 1 mL of extract solution. Bacteria or cell is splitted by ultrasonication(Power: 300 W, work time 3s, interval 7s, total time: 3 minutes). Centrifuge at 12000

rpm for 10 minutes at 4℃, take the supernatant and place it on ice for test.

3. Liquid samples: direct measurement.

II. Procedure

1. Preheat spectrophotometer for 30 minutes, adjust wavelength to 340 nm, set zero with distilled water.

2. Preheat Reagent II, Reagent III and Reagent IV at 37℃ for 10 minutes.(Preheat as much reagent as needed).

3. Procedure test

Reagent (μL)	Test tube (T)	Blank tube (B)
Reagent II	300	300
Reagent III	300	300
Reagent IV	300	300
Sample	100	-
Distilled water	-	100
Add reagents to 1 mL micro quartz cuvette orderly, mix thoroughly. Detect the absorbance at 340 nm at the time of 10 seconds record as A1. Then place dishes with the reaction solution in a 37℃ water bath or incubator for 10 minutes. Take it out and wipe it clean, immediately measure the absorbance at the time of 610 seconds which record as A2. $\Delta A_T=A_{T1}-A_{T2}$,$\Delta A_B=A_{B1}-A_{B2}$, $\Delta A=\Delta A_T-\Delta A_B$. The Blank tube only needs to be measured one or twice.		

III. Calculations of δ- OAT activity：

1. Protein concentration:

Unit definition: One unit of δ - OAT activity is defined as the amount of enzyme that per milligram of protein oxidation 1 mmoL of NADH per minute in the reaction system.

δ - OAT (U/minute prot)= $\Delta A\times V_{RV}\div(\varepsilon\times d)\times 10^9\div(V_S\div Cpr)\div T= 160.77\times\Delta A\div Cpr$

2. Sample weight:

Unit definition: One unit of δ - OAT activity is defined as the amount of enzyme that per gram of tissue oxidation 1 mmoL of NADH per minute in the reaction system.

δ -OAT (U/minute fresh weight)= $\Delta A\times V_{RV}\div(\varepsilon\times d)\times 10^9\div(W\times V_S\div V_E)\div T= 160.77\times\Delta A\div W$

3. Cell amount:

Unit definition: One unit of δ - OAT activity is defined as the amount of enzyme that per 10 thousand germ or cells oxidation 1 mmoL of NADH per minute in the reaction system.

δ -OAT (U/minute fresh weight)= $\Delta A\times V_{RV}\div(\varepsilon\times d)\times 10^9\div(V_S\div N\div V_E)\div T= 160.77\times\Delta A\div N$

4. Liquid volume:

Unit definition: One unit of δ - OAT activity is defined as the amount of enzyme that per milliliter of liquid oxidation 1 mmoL of NADH per minute in the reaction system.

δ-OAT（U/mL）=ΔA÷（ε×d）×V 反总×10^9÷V 样÷T= 160.77×ΔA

V_{RV}: Total reaction volume, 0.001 L;

ε: Molar extinction coefficient, 6.22×10^3L/mol/cm;

d: Cuvette light diameter(cm), 1 cm;

Vs: Sample volume, 0.1 mL;

$V_{E:}$ Extract solution volume, 1 mL;

T: Reaction time(min), 10 minutes;

Cpr: Sample protein concentration, mg/mL;

N: Total number of bacteria/cells, 10 million as a unit;

W: Sample weight, g.

Note:

1. If ΔA>0.5, please dilute the sample to appropriate concentration, multiply dilute times in the formular. If ΔA is too small, increase the sample volume or prolong the enzymatic reaction time.
2. After adding the reagents in turn, mixed as quickly as possible and measured the OD, to reduce the error time.
3. ΔA_B generally does not exceed 0.05.

Experimental Examples:

1. 1. Take 0.1g red bean stalks, carry out sample processing, and measure according to the operation steps. The calculation is: ΔAt=At1-At2=0.076, ΔAb=Ab1-Ab2=0, ΔA=ΔAt -ΔAb = 0.076, calculate the enzyme activity according to the sample weight:
   δ-OAT（U/g weight）=160.77×ΔA÷W÷d=160.77×0.076÷0.1÷1=122.19 U/g weight

Related Products:

NA0744/NA0502	Glutamic-pyruvic Transaminase(GPT) Activity Assay Kit
NA0743/NA0501	Glutamic-oxalacetic Transaminase(GOT) Activity Assay Kit
NA0341/NA0340	Proline Dehydrogenase(ProDH) Activity Assay Kit
NA0344/NA0345	Leucine Arylamidase(LAP) Activity Assay Kit