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Tannase Activity Assay Kit

Note: It is necessary to predict 2-3 large difference samples before the formal determination.

Operation Equipment: Spectrophotometer

Cat No: AK0356

Size: 50T/24S

Components:

Extract solution: Liquid 75 mL×2, store at 4℃;

Reagent I: Powder×1, store at 4℃ . Add 3 mL absolute ethanol **(self-provided reagent)** when the solution will be used. Mix thoroughly;

Standard: Powder×1, store at 4℃ . 5mg propyl gallate. Add 1.178 mL absolute ethanol when the solution will be used. Mix thoroughly. Form the standard solution of 20 μmol/mL.

Product Description:

Tannase is found in tannin rich plants and also in microorganisms. It can hydrolyze the ester bond and the phenol carboxyl bond to form gallic acid and glucose.

Using propyl gallate (PG) as the substrate of tannase, it has a characteristic absorption peak at 270nn. Tannase activity can be calculated by measuring the absorbance at 270 nm.

Required but Not Provided:

Ultraviolet spectrophotometer, desk centrifuge, water-bath, transferpettor, 1 mL quartz cuvette, absolute ethanol, mortar /homogenizer, ethanol, ice and distilled water.

Protocol

I. Preparation:

1. Tissue:

Add 1 mL of extract solution to 0.1 g of tissue. Homogenate on ice. Centrifuge at 10000 rpm 4℃ for 10 minutes. Take the supernatant on ice for test.

2. Cells or bacterial

Collect bacteria or cells into the centrifuge tube. Discard the supernatant after centrifugation. It is suggested to take about 5 million bacteria/cell and add 1 mL extract solution. Bacteria/cell is split by ultrasonication (power 20%, ultrasonic 3s, interval 10s, repeat for 30 times). Centrifuge at 10000 rpm 4℃ for 10 minutes. Take the supernatant on ice for test.

II. Determination procedure:

1. Preheat spectrophotometer for 30 minutes, adjust wavelength to 270 nm, set the counter to zero with distilled water.

2. Dilute the standard solution of 20 μmol/mL with the extract solution to 0.05 μmol/mL, and determine its absorbance at 270 nm, record as A_S .

3．Pipette 0.1 mL of the sample into a 1.5 mL EP tube as the control tube. Boil it for 5 min, and cool to room temperature.

4．Operation table: add the following reagents into 1.5mL EP tube respectively

Reagent Name (μL)	Test tube (A_T)	Control tube (A_C)
Extract solution	850	850
Reagent I	50	50
Sample	100	100 (deactivated)
After reaction in water bath at 40℃ for 10 min, boiling water bath immediately for 5 min. Centrifuge at room temperature of 10000 rpm for 10 min after cooling. Take the supernatant.		
Supernatant	50	50
Extract solution	950	950

Mix thoroughly. Measure the absorbance of 270 nm. Record as A_T, A_C. $\Delta A = A_C - A_T$.

III. Tannase Calculation:

1. Protein concentration:

Unit definition: One unit of enzyme is defined as the amount of enzyme that catalyzes the consumption of 1 nmol PG per minute every mg tissue protein in the reaction system.

Tannase (U/mg prot)$= \Delta A \div (A_S \times C_S) \times 1000 \times F \times V_R \div (Cpr \times V_S) \div T$

$\qquad = 1000 \times \Delta A \div Cpr \div A_S$

2. Sample weight:

Unit definition: One unit of enzyme is defined as the amount of enzyme that catalyzes the consumption of 1 nmol PG per minute every gram tissue weight in the reaction system.

Tannase (U/g weight)$= \Delta A \div (A_S \times C_S) \times 1000 \times F \times V_R \div (V_S \times W \div V_E) \div T$

$\qquad = 1000 \times \Delta A \div A_S \div W$

3. Cells or bacterial

Unit definition: One unit of enzyme is defined as the amount of enzyme that catalyzes the consumption of 1 nmol PG per minute every 10^4 cells or bacterial in the reaction system.

Tannase (U/10^4 cell)$= \Delta A \div (A_S \times C_S) \times 1000 \times F \times V_R \div (V_S \times 500 \div V_E) \div T$

$\qquad = 2 \times \Delta A \div A_S$

C_S : Concentration of standard solution, 0.05 μmol/mL;

F: Dilution ratio of supernatant, F= 1 mL÷0.05 mL=20;

1000：1 μmol=1000 nmol；

V_S : Sample volume, 0.1 mL;

V_R : Total reaction volume, 1 mL;

Cpr: Protein concentration, mg/mL;

W: Sample weight, g;

V_E : Extract solution volume of cells, 1 mL;

500: Cells or germ, 5 million;

T: Reaction time, 10 minutes;

Note:

1. If the A_T is greater than 1.5. Increase the dilution ratio appropriately. But the total volume of 1 mL should be kept unchanged. For example, add 20 µL supernatant and 980 µL reagent I (equivalent to F= 1000/20 = 50). The value of F and V_S should be changed in the calculation formula.

Experimental Examples:

1. Take 0. 1g of *Yulania denudata* leaf and add 1mL extract to homogenize and grind, take the supernatant and operate according to the determination steps, and calculate ΔAt = Ac-At= 1. 126-1.033=0.093, calculate the enzyme based on the sample weight:

Tannase Activity（U/g weight）= 1000×ΔA÷As÷W=1000×0.093÷0.609÷0. 1=1527.09 U/g weight。

Related Products:

AK0446/AK0445 Uric Acid(UA) Content Assay Kit

AK0450/AK0449 Plant Total Phenol Content Assay Kit

AK0452/AK0451 Plant Flavonoids Content Assay Kit