Tannase Activity Assay Kit

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

Operation Equipment: Spectrophotometer/microplate reader

Cat No: BC4075

Size: 100T/48S

Components:

Extract solution: Liquid 120 mL×1, store at 4℃;

Reagent I: Powder×2, store at 4℃. Add 1 mL absolute ethanol when the solution will be used. Mix thoroughly. It could be stored at 4℃ for one week.

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. It could be stored at 4℃ for two weeks.

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/microplate reader, desk centrifuge, water-bath, transferpettor, micro quartz cuvette/96 well flat-bottom UV plate, mortar/homogenizer, ethanol, ice and distilled water.

Protocol

I. Preparation:

1. Tissue:

Add 1 mL of extraction reagent 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 extraction reagent. Bacteria/cell is split by ultrasonication (power 200W, 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 ultraviolet spectrophotometer/microplate reader for 30 minutes, adjust wavelength to 270 nm, set spectrophotometer counter to zero with distilled water.

2. Standard working solution: dilute the standard solution of 20 μmol/mL with the extraction

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solution to 0.05 μmol/mL, and determine its absorbance at 270 nm, record as A_S.

3. Pipette 0.02 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 1.5 mL EP tube respectively

Reagent (μL)	Test tube (A_T)	Control tube (A_C)
Extract solution	170	170
Reagent I	10	10
Sample	20	20(deactivated)
After reaction in water bath at 40°C 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. Add the following reagents to the micro quartz cuvette/96 well UV plate respectively:		
Supernatant	10	10
Extract solution	190	190

Mix thoroughly. Measure the absorbance of 270 nm. Record as A_T, A_C. $\Delta A=A_C-A_T$. Each test tube should be provided with one contrast tube. Standard curve and blank tube only need to be measured once or twice.

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)=$\Delta A\div(A_S\times C_S)\times1000\times F\times V_R\div(Cpr\times V_S)\div T=1000\times\Delta A\div Cpr\div A_S$

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)=$\Delta A\div(A_S\times C_S)\times1000\times F\times V_R\div(V_S\times W\div V_E)\div T=1000\times\Delta A\div A_S\div W$

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)=$\Delta A\div(A_S\times C_S)\times1000\times F\times V_R\div(V_S\times500\div V_E)\div T=2\times\Delta A\div A_S$

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

F: Dilution ratio of supernatant, F= 200 μL÷10 μL = 20;

1000：1 μmol=1000 nmol;

V_S: Sample volume, 0.02 mL;

V_R: Total reaction volume, 0.2 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 5 μL supernatant and 195 μL reagent I (equivalent to F= 200/5 = 40). 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, measure by the micro quartz cuvette and calculate ΔAt = Ac-At=0.6631-0.6258=0.0373, calculate the enzyme based on the sample weight：:

Tannase Activity（U/g weight）=1000×ΔA÷A_S÷W×F（dilute times）=1119 U/g weight。

Related Products:

BC1360/BC1365 Uric Acid(UA) Content Assay Kit

BC1340/BC1345 Plant Total Phenol Content Assay Kit

BC1330/BC1335 Plant Flavonoids Content Assay Kit

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