

# 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:** NA0358

**Size:** 100T/48S

## Components:

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

Reagent I: Powder×1, store at 4°C. Add 2 mL absolute ethanol when the solution will be used. Mix thoroughly;

Standard: Powder×1, store at 4°C. 5mg propyl gallate. Add 1.178 mL absolute ethanol (**self-provided reagent**) 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 270nm. 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°C 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 20%, ultrasonic 3s, interval 10s, repeat for 30 times). Centrifuge at 10000 rpm 4°C 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 the counter to zero with distilled water.

2. Dilute the standard solution of 20 μmol/mL with the extraction 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 Name ( $\mu\text{L}$ )	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 $40^\circ\text{C}$ 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  $A_T$ ,  $A_C$ .  $\Delta A = A_C - A_T$ .

### 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.

$$\text{Tannase (U/mg prot)} = \Delta A \div (A_S \times C_S) \times 1000 \times F \times V_R \div (C_{pr} \times V_S) \div T = 1000 \times \Delta A \div C_{pr} \div A_S$$

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.

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

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.

$$\text{Tannase (U/10}^4 \text{ cell)} = \Delta A \div (A_S \times C_S) \times 1000 \times F \times V_R \div (V_S \times 500 \div V_E) \div T = 2 \times \Delta A \div A_S$$

$C_S$ : Concentration of standard solution,  $0.05 \mu\text{mol/mL}$ ;

F: Dilution ratio of supernatant,  $F = 200 \mu\text{L} \div 10 \mu\text{L} = 20$ ;

1000:  $1 \mu\text{mol} = 1000 \text{ nmol}$ ;

$V_S$ : Sample volume,  $0.02 \text{ mL}$ ;

$V_R$ : Total reaction volume,  $0.2 \text{ mL}$ ;

$C_{pr}$ : Protein concentration,  $\text{mg/mL}$ ;

W: Sample weight, g;

$V_E$ : Extract solution volume of cells,  $1 \text{ mL}$ ;

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  $\mu\text{L}$  supernatant and 195  $\mu\text{L}$  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  $\Delta A_t = A_c - A_t = 0.6631 - 0.6258 = 0.0373$ , calculate the enzyme based on the sample weight: :

Tannase Activity (U/g weight) =  $1000 \times \Delta A \div A_{\text{标准}} \div W \times F$  (dilute times) = 1119 U/g weight。

**Related Products:**

NA0763/NA0522 Uric Acid(UA) Content Assay Kit

NA0765/NA0524 Plant Total Phenol Content Assay Kit

NA0766/NA0525 Plant Flavonoids Content Assay Kit