CheKine™ Micro Tannase (TAN) Activity Assay Kit

Cat #: KTB1542 Size: 48 T/96 T

	Micro Tannase (TAN) Activity Assay Kit		
REF	**Cat #:** KTB1542	LOT	**Lot #:** Refer to product label
	Detection range: 0.3125-20 μmoL/mL (The detection range corresponds to the standard)		**Sensitivity:** 0.156 μmoL/mL (The sensitivity corres -ponds to the standard)
	Applicable samples: Plant Tissues, Fungi, Bacteria		
	Storage: Stored at 4℃ for 6 months, protected from light		

Assay Principle

Tannase, full name is Tannin Acyl Hydrolase (Tannase, EC 3.1.1.20). Tannase exists in tannin-rich plants and also widely exists in microorganisms. Tannase hydrolyses the ester bonds and depside bonds in gallic acid tannins to release gallic acid and glucose. The enzyme can be produced by molds such as Aspergillus Niger, Aspergillus oryzae. It can be used to treat tannin and protein in beer to make it clear and transparent. It can also be used to remove the astringency of persimmon and other products. And it can also be used to make instant tea to prevent turbid fermented tea. CheKine™ Micro Tannase (TAN) Activity Assay Kit provides a convenient tool for detection of Tannase activity. The principle is to use propyl gallate (PG) as the substrate for the enzymatic reaction of tanninase, which has a characteristic absorption peak at 270 nm. The Tannase activity measure the change of the absorbance at 270 nm before and after the reaction, and calculate the Tannase activity of the sample can be calculated by measuring the absorbance at 270 nm.

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	48 T	96 T	
Extraction Buffer	100 mL	100 mL×2	4℃
Substrate	5 mL	10 mL	4℃, protected from light
Standard	1	2	4℃, protected from light

Materials Required but Not Supplied

- Microplate reader or ultraviolet spectrophotometer capable of measuring absorbance at 270 nm
- 96-well UV plate or microquartz cuvette, precision pipettes, disposable pipette tips
- Water bath, ice maker, refrigerated centrifuge
- Anhydrous ethanol
- Homogenizer (for tissue samples)

Reagent Preparation

Extraction Buffer: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃.

Substrate: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃, protected from light.

Standard: Add 1.178 mL of anhydrous ethanol to dissolve before use. The concentration is 20 μmol/mL. This solution can be stored at 4℃ for one week or stored at -20℃ for long time.

Standard curve setting: Dilute 20 μmol/mL Standard with Extraction Buffer to 10, 5, 2.5, 1.25, 0.625, 0.313 μmol/mL Standard solution as shown in the table below.

Num.	Volume of Standard	Volume of Extraction Buffer (μL)	The Concentration of Standard (μmol/mL)
Std.1	200 μL of 20 μmol/mL	0	20
Std.2	100 μL of Std.1 (20 μmol/mL)	100	10
Std.3	100 μL of Std.2 (10 μmol/mL)	100	5
Std.4	100 μL of Std.3 (5 μmol/mL)	100	2.5
Std.5	100 μL of Std.4 (2.5 μmol/mL)	100	1.25
Std.6	100 μL of Std.5 (1.25 μmol/mL)	100	0.625
Std.7	100 μL of Std.6 (0.625 μmol/mL)	100	0.313

Sample Preparation

Note: Fresh samples are recommended, If not assayed immediately, samples can be stored at -80℃ for one month. Processed samples must be assayed immediately. It will be better to quantify the total protein with Protein Quantification Kit (BCA Assay), Cat #: KTD3001, if the content is calculated by protein concentration.

1. Plant tissues: Weigh 0.1 g tissue, add 1 mL Extraction Buffer and homogenize on ice. Centrifuge at 10,000 g for 10 min at 4℃. Use supernatant for assay, and place it on ice to be tested.

2. Fungi or bacteria: Collect 5×10^6 fungi or bacteria into the centrifuge tube, wash fungi or bacteria with cold PBS, discard the supernatant after centrifugation; add 1 mL Extraction Buffer to ultrasonically disrupt the fungi or bacteria in ice bath 5 min (power 20% or 200 W, ultrasonic 3 s, interval 7 s, repeat 30 times). Centrifuge at 10,000 g for 10 min at 4℃. Use supernatant for assay, and place it on ice to be tested.

Assay Procedure

1. Preheat the microplate reader or ultraviolet spectrophotometer for more than 30 min, and adjust the wavelength to 270 nm, ultraviolet spectrophotometer was returned to zero with deionized water.
2. Add 50 μL of sample into an EP tube as a control tube, bath in boiling water for 5 min, and cool to room temperature.
3. Add the following reagents respectively into each EP tube:

Reagent	Blank Tube (μL)	Standard Tube (μL)	Test Tube (μL)	Control Tube (μL)
Extraction Buffer	200	150	100	100
Stds.	0	50	0	0
Sample	0	0	50	50 (deactivated)
Substrate	0	0	50	50

Mix well, incubate at 40℃ water bath for 10 min, and then immediately take a boiling water bath for 5 min. After cooling, centrifuge at 10,000 g at room temperature (25℃) for 10 min, and take the supernatant.

Add the following reagents to a 96-well UV plate or microquartz cuvette:

Supernatant	10	10	10	10
Extraction Buffer	190	190	190	190

4. Mix well, read the values at 270 nm. Recorded as A_{Blank}, $A_{Standard}$, A_{Test} and $A_{Control}$, respectively. Finally, calculate $\Delta A_{Test}=A_{Control}-A_{Test}$, $\Delta A_{Standard}=A_{Standard}-A_{Blank}$ (only one blank well needs to be detected, a control is required for each sample).

Note: In order to guarantee the accuracy of experimental results, need to do a pre-experiment with 2-3 samples. If ΔA_{Test} is greater than 1.0, the sample can be appropriately diluted with Extraction Buffer, the calculated result multiplied by the dilution factor. If ΔA_{Test} is less than 0.001, increase the sample quantity appropriately.

Data Analysis

Note: We provide you with calculation formulae, including the derivation process and final formula. The two are exactly equal. It is suggested that the concise calculation formula in bold is final formula.

1. Drawing of standard curve

With the concentration of the standard solution as the y-axis and the $\Delta A_{Standard}$ as the x-axis, draw the standard curve. Substitute the ΔA_{Test} into the equation to obtain the y value (μmol/mL).

2. Calculate the activity of Tannase (TAN)

(1) By sample weight

Unit definition: 1 nmol PG reduced per min in 1 g tissue reaction system at 40℃ is defined as a unit of enzyme activity.

$TAN\ (U/g)=y\times1,000\times F\times V_{Reaction\ Total}\div(W\times V_{Sample}\div V_{Sample\ Total})\div T\times n$**$=8,000\times y\div W\times n$**

(2) By cells number of fungi or bacteria

Unit definition: 1 nmol PG reduced per min in 10^4 number of fungi or bacteria reaction system at 40℃ is defined as a unit of enzyme activity.

$TAN\ (U/10^4)=y\times1,000\times F\times V_{Reaction\ Total}\div(V_{Sample}\times500\div V_{Sample\ Total})\div T\times n$**$=16\times y\times n$**

(3) By protein concentration

Unit definition: 1 nmol PG reduced per min in 1 mg tissue protein reaction system at 40℃ is defined as a unit of enzyme activity.

$TAN\ (U/mg\ prot)=y\times1,000\times F\times V_{Reaction\ Total}\div(Cpr\times V_{Sample})\div T\times n$**$=8,000\times y\div Cpr\times n$**

Where: 1,000: 1 μmol=1,000 nmol; F: dilution factor of supernatant, F=200 μL÷10 μL=20; $V_{Reaction\ Total}$: total reaction volume, 0.2 mL; W: sample weight, g; V_{sample}: sample volume added, 0.05 mL; $V_{Sample\ Total}$: Extraction Buffer volume added, 1 mL; T: reaction time, 10 min; n: dilution multiple of sample; 500: total number of fungi or bacteria, 5×10^6; Cpr: sample protein concentration, mg/mL.

Typical Data

Typical standard curve:

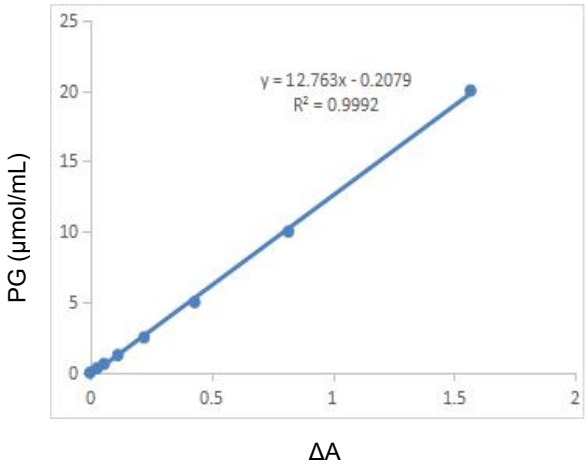

Figure 1. Standard Curve for PG.

Recommended Products

Catalog No.	Product Name
KTB1540	CheKine™ Micro Plant Total Phenols (TP) Assay Kit
KTB1530	CheKine™ Micro Plant Flavonoids Assay Kit
KTB1541	CheKine™ Micro Tannin Assay Kit

Disclaimer

The reagent is only used in the field of scientific research, not suitable for clinical diagnosis or other purposes.