CheKine™ Micro Chitinase Activity Assay Kit

Cat #: KTB3012 Size: 48 T/24 S 96 T/48 S

	Micro Chitinase Activity Assay Kit		
REF	**Cat #:** KTB3012	LOT	**Lot #:** Refer to product label
	Detection range: 0.15-1.6 mg/mL		**Sensitivity:** 0.15 mg/mL
	Applicable samples: Animal Tissue, Fungus, Liquid samples		
	Storage: Stored at 4℃ for 6 months, protected from light		

Assay Principle

Chitin is mainly found in the shells of crustaceans such as shrimp, crabs, insects and other crustaceans and the organs of molluscs (such as the cartilage of cuttlefish), as well as in the cell walls of fungi. Chitinase (EC3.2.1.14) can catalyze chitin hydrolysis, which has the effect of resisting fungal infection and has become a hot topic in research against fungal diseases. CheKine™ Micro Chitinase Activity Assay Kit can be used to detect biological samples such as animal tissue, fungus, liquid samples. In the kit, chitinase hydrolyzes chitin to produce N-acetyl-D-glucosamine, which further reacts with DNS reagent to produce a brown-red compound. There is a characteristic absorption peak at 540 nm, and the rate of increasing absorption value reflects the activity of chitinase.

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	48 T	96 T	
Extraction Buffer	60 mL	120 mL	4℃
Reagent Ⅰ	3 mL	6 mL	4℃
Reagent Ⅱ	6 mL	12 mL	4℃, protected from light
Reagent Ⅲ	10 mL	20 mL	4℃, protected from light
Standard	Powder×1 vial	Powder×1 vial	4℃, protected from light

Note: Before formal testing, it is recommended to select 2-3 samples with large expected differences for pre-experiment.

Materials Required but Not Supplied

- Microplate reader or visible spectrophotometer capable of measuring absorbance at 540 nm
- 96-well plate or microglass cuvette, precision pipettes, disposable pipette tips, 1.5 mL EP tube
- Incubator, ice maker, freezing centrifuge

• Deionized water
• Homogenizer or mortar (for tissue samples)

Reagent Preparation

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

Reagent I: Ready to use as supplied; Equilibrate to room temperature before use; Store at 4℃.

Reagent II: Ready to use as supplied; Equilibrate to room temperature before use; Store at 4℃, protected from light. Reagent II is a saturated solution, shake well before use.

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

Note: Reagent III has certain irritation, so personal protection is recommended during use.

Standard: Prepared before use. Add 1 mL deionized water for each bottle to fully dissolve, that is 5 mg/mL N-Acetyl-D-glucosamine Standard. Equilibrate to room temperature before use; Store at 4℃, protected from light for 1 month. Using 5 mg/mL N-Acetyl-D-glucosamine Standard, prepare standard curve dilution as described in the table:

Num.	Standard Volume (μL)	Deionized water (μL)	Concentration (mg/mL)
Std.1	80 μL of 5 mg/mL Standard	170	1.6
Std.2	70 μL of 5 mg/mL Standard	180	1.4
Std.3	60 μL of 5 mg/mL Standard	190	1.2
Std.4	50 μL of 5 mg/mL Standard	200	1.0
Std.5	40 μL of 5 mg/mL Standard	210	0.8
Std.6	30 μL of 5 mg/mL Standard	220	0.6
Std.7	20 μL of 5 mg/mL Standard	230	0.4
Std.8	7.5 μL of 5 mg/mL Standard	242.5	0.15

Note: Always prepare fresh standards per use; Diluted Standard Solution is unstable and must be used within 4 h.

Sample Preparation

Note: We recommend that you use fresh samples. If not assayed immediately, samples can be stored at -80℃ for one month.When measuring, the temperature and time of thawing should be controlled. When thawing at room temperature, the sample should be thawed within 4 h.

1. Plant tissues: Weigh 0.1 g tissue, add 1 mL Extraction Buffer and homogenize or mortar on ice. Centrifuge at 10,000 g for 20 min at 4℃. Use supernatant for assay, and place it on ice to be tested.
2. Fungus: Collect 5×10^6 fungus into the centrifuge tube, wash fungus with cold PBS, discard the supernatant after centrifugation; add 1 mL Extraction Buffer to ultrasonically disrupt the fungus 5 min (power 20% or 200 W, ultrasonic 3 s, interval 7 s, repeat 30 times). Centrifuge at 10,000 g for 20 min at 4℃. Use supernatant for assay, and place it on ice to be tested.
3. Liquid samples: Test directly. If the solution is turbid, centrifuge at 150,000 g for 20 min at 4℃. Use supernatant for assay, and place it on ice to be tested.

Note: If the protein concentration of the sample is need to determined, it is recommended to use Abbkine Cat #: KTD3001 Protein Quantification Kit (BCA Assay) to measure the protein concentration of the sample.

Assay Procedure

1. Preheat the microplate reader or visible spectrophotometer for more than 30 min, and adjust the wavelength to 540 nm. Visible spectrophotometer was returned to zero with deionized water.

2. Sample measurement. (The following operations are operated in 96-well plate or microglass cuvette)

Reagent	Test Tube (μL)	Control Tube (μL)	Standard Tube (μL)	Blank Tube (μL)
Sample	100	0	0	0
Inactivated sample (10 min for boiling water bath)	0	100	0	0
Reagent Ⅰ	50	50	0	0
Reagent Ⅱ	100	100	0	0
Mix well and incubate at 37℃ for 1 h. The reaction was terminated in a boiling water bath for 5 min, and after cooling at room temperature, centrifuged at 8,000 g for 10 min at 4℃, use supernatant for assay.			0	0
Supernatant	175	175	0	0
Standard	0	0	175	0
Deionized water	0	0	0	175
Reagent Ⅲ	125	125	125	125

3. Mix well, accurate incubation at 95℃ for 5 min, take 200 μL into 96-well microplate or microglass cuvette after cooling at room temperature, detect the absorbance at 540 nm. The Blank Well is recorded as A_{Blank}, the Standard Well is marked as $A_{Standard}$, the Control Well is marked as $A_{Control}$, and the Test Well is marked as A_{Test}. Finally calculate $\Delta A_{Test}=A_{Test}-A_{Control}$, $\Delta A_{Standard}=A_{Standard}-A_{Blank}$.

Note: (1) The Standard Well and Blank Well only need to be done once or twice, Each Test Well needs to be provided with a Control Well. (2) In order to guarantee the accuracy of experimental results, need to do a pre-experiment with 2-3 samples. (3) If ΔA_{Test} is less than 0.01, increase the sample quantity appropriately. If ΔA_{Test} is greater than $\Delta A_{Standard}$ of 1.6 mg/mL, the sample can be appropriately diluted with Extraction Buffer, the calculated result multiplied by the dilution factor, or decrease 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 x-axis and the $\Delta A_{Standard}$ as the y-axis, draw the standard curve and obtain the standard equation. The determination of ΔA_{Test} is substituted into the equation to get x (mg/mL).

2. Calculation of the chitinase activity

(1) Calculated by protein concentration

Active unit definition: At 37℃, 1 mg N-Acetyl-D-glucosamine is produced by decomposing chitin per h in 1 mg tissue protein reaction system is defined as a unit of enzyme activity.

Chitinase (U/mg prot)=$(V_{Total}\times x)\div(V_{Sample}\times Cpr)\div T$**=2.5x÷Cpr**

(2) Calculated by fresh weight of samples

Active unit definition: At 37℃, 1 mg N-Acetyl-D-glucosamine is produced by decomposing chitin per h in 1 g tissue reaction system is defined as a unit of enzyme activity.

Chitinase (U/g fresh weight)=$(V_{Total}\times x)\div(W\times V_{Sample}\div V_{Total\ sample})\div T$**=2.5x÷W**

(3) Calculated by the number of fungus

Active unit definition: At 37℃, 1 mg N-Acetyl-D-glucosamine is produced by decomposing chitin per h in 10^4 fungus reaction system is defined as a unit of enzyme activity.

Chitinase (U/10^4 fungus)=$(V_{Total}\times x)\div(N\times V_{Sample}\div V_{Total\ sample})\div T$**=2.5x÷N**

(4) Calculated by volume of liquid samples

Active unit definition: At 37℃, 1 mg N-Acetyl-D-glucosamine is produced by decomposing chitin per h in 1 mL liquid samples reaction system is defined as a unit of enzyme activity.

Chitinase (U/mL)=(V_{Total}×x)÷V_{Sample}÷T**=2.5x**

V_{Total}: Total volume of reaction system, 0.25 mL; V_{Sample}: Added the sample volume, 0.1 mL; $V_{Total\ sample}$: Added the Extraction Buffer volume, 1 mL;T: Reaction time, 1 h; Cpr: sample protein concentration, mg/mL; W: Sample weight, g; N: Fungus counts in tens of thousands.

Typical Data

The following data are for reference only. And the experimenters need to test the samples according to their own experiments.

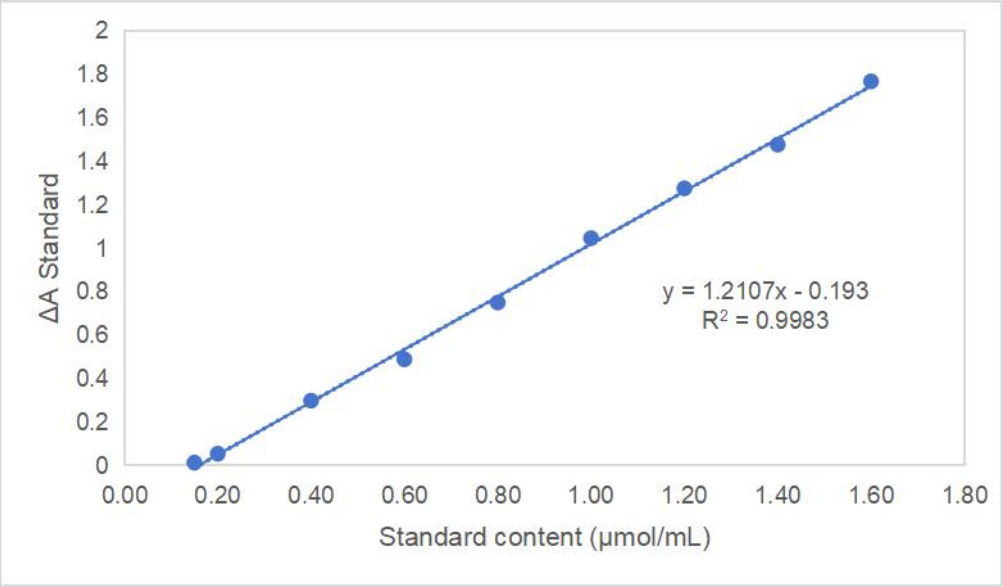

Figure 1. Standard curve of chitinase.

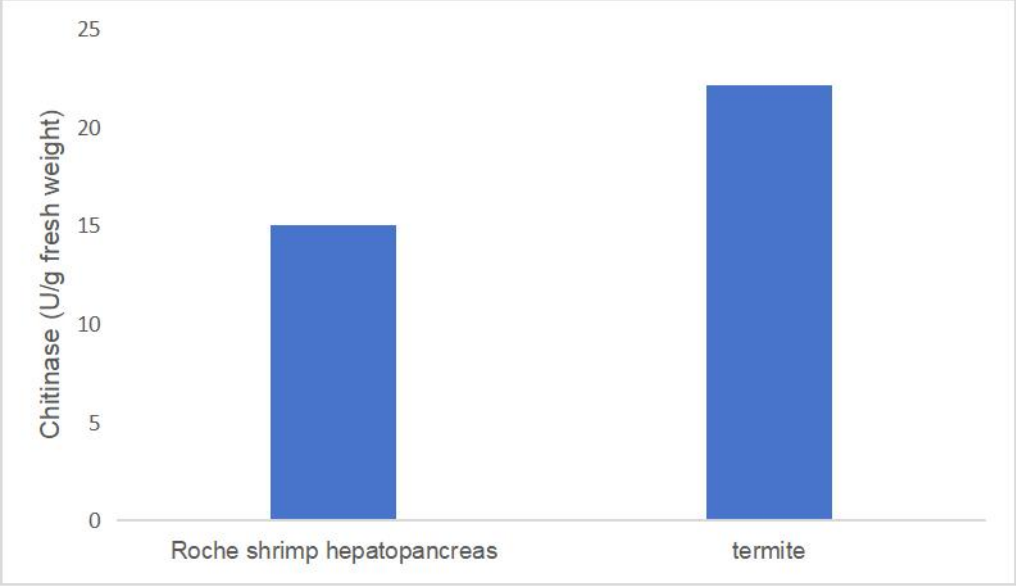

Figure 2. Determination of Chitinase activity in Roche shrimp hepatopancreas and termite by this kit.

Recommended Products

Catalog No.	Product Name

KTB1150	CheKine™ Micro Peroxidase (POD) Activity Assay Kit
KTB1030	CheKine™ Micro Superoxide Dismutases (SOD) Activity Assay Kit
KTB1040	CheKine™ Micro Catalase (CAT) Content Assay Kit

Disclaimer

The reagent is only used in the field of scientific research, not suitable for clinical diagnosis or other purposes. For your safety and health, please wear a lab coat and disposable gloves.