Chitinase Activity Assay Kit

Note: Take two or three different samples for prediction before test.

Operation Equipment: Spectrophotometer/Microplate reader

Cat No: BC0825

Size:100T/48S

Components:

Extract solution: 60 mL×1. Storage at 2-8℃;

Reagent I: 8mL×1. Storage at 2-8℃, shake well before use.

Reagent II: 9mL×1. Storage at 2-8℃. The reagent is a suspension, which needs to be shaken before use.

Reagent III：3mL×1. Storage at 2-8℃. The reagent is saturated, crystals will precipitate when taken out at low temperature (2-8℃), and it can be dissolved by heating at 60℃

Reagent IV A：Powder×2. Storage at 2-8℃.

Reagent IV B：50mL×1. Storage at 2-8℃.

Standard: powder×1, 5 mg N-acetylglucosamine, storage at 4℃. Add 1 mL of Reagent I before use, which is 5000μg/mL standard solution. The unused reagent can be stored at 4℃ for 2 weeks.

Reagent IV: Take a bottle of Reagent IV A, add it to 21mL of Reagent IV B, dissolve it fully. Unused reagents can be stored at 2-8℃ for 4 weeks; Reagent 4B can promote dissolution at 37 ℃ if it has crystallization.

Product Description:

Chitinases are found in the shells of crustaceans such as shrimps, crabs and insects, and in the organs of mollusks (such as squid cartilage), as well as in the cell walls of fungi. Chitinase (EC 3.2.1.14) can catalyze the hydrolysis of chitin, which has the function of resisting fungal infection and become the research hotspot of antifungal diseases.

Chitinase hydrolyzes chitin to produce N-acetylglucosamine, and the intermediate compound produced by co-heating of N-acetylglucosamine with alkali can further react with p-dimethylaminobenzaldehyde to produce a chromogenic substance, which has the characteristic absorption peaks at 585nm, and the rate of increase in absorbance reflects the activity of chitinase.

Reagents and Equipment Required but Not Provided:

Scales, water bath/constant temperature incubator, desk centrifuge, spectrophotometer/microplate reader, micro glass cuvette/96 well flat-bottom plate, mortar/homogenizer, distilled water, ultrasonic crusher.

procedure

1. Extraction of crude enzyme solution:

a. Tissue

The ratio of tissue mass (g): the volume of Extract solution(mL) is 1: 5~10 (it is suggested to take about 0.1 g tissue and add 1 mL Extract solution), ice-bath homogenate. Centrifuge at 10000 g for 20

minutes at 4℃, take the supernatant and placed on the ice for test.

b. Bacteria or cells

The ratio of bacteria/cell amount (10^4): the volume of Extract solution(mL) is 500~1000:1(it is suggested to take about 5 million bacteria/cell and add 1 mL of Extract solution). Bacteria/cell is split by ultrasonic (placed on ice, 300 W, work time 3 s，interval 7 s，the total time is 3 minutes). Centrifuge at 10000 g for 20 minutes at 4℃, take the supernatant and placed on the ice for test.

c. Serum (plasma) sample: Detect sample directly.

2. Determination procedure

a. Preheat the Spectrophotometer/Microplate reader 30 minutes, adjust wavelength to 540 nm. The spectrophotometer needs to be zeroed with distilled water.

b. standard solution

Dilute the 5000μg/mL standard solution with distilled water to 125、62.5、31.25、15.625、7.8125、3.9、1.953、0.976、0.488μg/mL standard solution for use.

c. Then operate according to the following table.

Reagent（μL）	Contrast Tube（A_C）	Test tube（A_T）	Blank Tube（A_B）	Standard Tube（A_S）
Sample	80	80	-	-
Reagent I	40	40	-	-
Reagent II	80	80	-	-
The enzymatic reaction was carried out at 37°C for 1 hour, followed by a boiling water bath for 5 minutes. Centrifuge at 10,000 rpm for 5 min at room temperature and take the supernatant			-	-
supernatant	100	100	-	-
Reagent I	-	-	100	-
standard solution	-	-	-	100
Reagent III	20	20	20	20
Mix well and let stand at room temperature for 5min		Mix well, take a boiling water bath for 5 min, and cool to room temperature with running water		
Reagent IV	300	300	300	300

Mix well, incubate at 37°C for 20 min, pipette 200 μL into a micro glass cuvette/96-well plate to measure the absorbance at 585 nm, and record as A_C, A_T, A_B, and A_S respectively. Calculate $\Delta A_T = A_T - A_C$, $\Delta A_S = A_S - A_B$. (The standard curve and blank tube only need to be measured 1-2 times)

Calculation formula

1. Drawing of standard curve

Take ΔA_S as y-axis, take standard solution concentration as x-axis, draw standard curve, get standard equation y=kx+b, bring ΔA_T into equation to get x (μg/mL)

2. Calculate the activity of Chitinase

(1) Calculated by sample weight

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the decompose chitin to produce 1 μmol of N-acetylglucosamine per hour at 37℃ every gram tissue.

Chitinase activity（U/g fresh wight）=$x \times V_E \div (V_S \div V_{ST} \times W) \div T = 2.5x \div W$。

(2) Calculated by Protein concentration:

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the decompose chitin to produce 1 μmol of N-acetylglucosamine per hour at 37℃ every milligram protein.

Chitinase activity（U/mg prot）= $x \times V_E \div (V_S \times Cpr) \div T = 2.5x \div Cpr$

(3) Calculated by Cell amount

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the decompose chitin to produce 1 μmol of N-acetylglucosamine per hour at 37℃ every 10000 cells.

Chitinase activity（$U/10^4$ cell）=$x \times V_E \div (V_S \div V_{ST} \times N) \div T = 2.5x \div N$

(4) Calculated by the volume of culture medium

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the decompose chitin to produce 1 μmol of N-acetylglucosamine per hour at 37℃ every milliliter of culture medium.

Chitinase activity (U/mL) = $x \times V_E \div V_S \div T = 2.5x$.

V_E：Total volume of enzymatic reaction，0.2mL；

V_S: Add the volume of sample, 0.08 mL;

V_{ST}: The volume of extract, 1 mL;

T: Reaction time, 1 hour;

Cpr: Sample protein concentration, mg/mL;

W: Sample weight, g;

N: Total number of bacteria or cells, 10^4 cells as a unit;

Note:

1. Color comparison shall be carried out as soon as possible after the reaction.
2. If the OD value is greater than 1.5, the sample shall be diluted properly and then determined. Pay attention to multiply the dilution ratio in the calculation formula; or shorten the water bath time of 37℃ to X hours (such as 0.5 hours), and divide the result by X according to the original calculation formula.

Experimental example:

1. Take 0.1g of shrimp shell and add 1 mL of Extract solution for homogenate grinding. After taking

supernatant, operate according to the determination steps. Use 96 well plate to measure and calculate $\Delta A_T=A_T-A_C$= 0.531-0.054=0.477, bring in standard curve y = 0.007x+0.0205, and get x =65.214μg/mL.

Chitinase activity (U/g mass) =2.5×x÷W=1468.790U/g mass.

2. Take 0.1g of Oyster mushroom and add 1 mL of Extract solution for homogenate grinding. After taking supernatant, operate according to the determination steps. Use 96 well plate to measure and calculate $\Delta A_T=A_T-A_C$=0.754-0.055=0.699, bring in standard curve y = 0.007x+0.0205, and get x =96.929 μg/mL.

   Chitinase activity (U/g mass) =2.5×x÷W=2144.437 U/g mass.

Related Products:

BC0230/BC0235	Reducing Sugar(RS) Content Assay Kit
BC2600/BC2605	Acidic Xylanase Activity Assay Kit
BC2550/BC2555	α-glucosidase(α-GC) Activity Assay Kit
BC2620/BC2625	β-xylosidase Activity Assay Kit