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Chitinase Activity Assay Kit

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

Operation Equipment: Spectrophotometer/Microplate reader

Cat No: AK0266

Size:100T/48S

Components:

Extract solution: 50 mL×1. Storage at 4℃;

Reagent I: 15 mL×1. Storage at 4℃, shake well before use.

Reagent II: 5 mL×1. Storage at 4℃ .

Standard: powder×1, 5 mg N-acetylglucosamine, storage at 4℃ . Add 2.27 mL of distilled water before use, which is 10 μmol/mL standard solution.

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 further reacts with 3,5-Dinitrosalicylic acid to produce brownish red compound. The brownish red compound has a characteristic absorption peak at 540 nm, and the increase rate of absorption value reflects the activity of chitinase.

Reagents and Equipment Required but Not Provided:

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

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, set zero with distilled water.

b. standard solution

Dilute the 10 μmol/mL standard solution with distilled water to 5, 4, 3, 2, 1 μmol/mL standard solution for use.

c. Then operate according to the following table.

Reagent name(μL)	Test tube(T)	Control tube(C)	Standard tube(S)	Blank tube(B)
sample	100	100	-	-
standard solution	-	-	100	-
Distilled water	-	-	-	100
Reagent Ⅰ	100	-	100	100
Mix well, water bath at 37℃ for 1 hour, boiling water bath for 5 minutes.				
Reagent Ⅰ	-	100	-	-
Centrifuge at 8000 rpm for 10 minutes at room temperature, take 160 μL of supernatant respectively and put it into a new EP tube, then add the following reagents				
Reagent Ⅱ	40	40	40	40
Mix well, react in boiling water bath for 10 minutes, and immediately put it on ice to room temperature. Determine the absorbance of each tube at 540 nm in micro glass cuvette/96 well flat-bottom plate, and record it as A_T, A_C, A_S and A_B. Calculate $\Delta A_T=A_T-A_C$, $\Delta A_S=A_S-A_B$.				

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 (μmol/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 ofN-acetylglucosamine per hour at 37℃ every gram tissue.

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

(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 ofN-acetylglucosamine per hour at 37℃ every milligram protein.

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

(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 ofN-acetylglucosamine per hour at 37℃ every 10000 cells.

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

(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 ofN-acetylglucosamine per hour at 37℃ every milliliter of culture medium.
Chitinase activity (U/mL) = $x \times V_S \div V_S \div T = x$.

V_S : Add the volume of sample, 0.1 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 ;

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:

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 $\Delta A_T = A_T - A_C$ = 0.716-0.644 = 0.072, bring in standard curve y = 0.244x-0. 1628, and get x = 0.9623.
Chitinase activity (U/g mass) = x ÷ W = 0.9623 ÷ 0.1 = 9.623 U/g mass.

Related Products:

AK0315/AK0314	Reducing Sugar(RS) Content Assay Kit
AK0199/AK0198	Acidic Xylanase Activity Assay Kit
AK0209/AK0208	α-glucosidase(α-GC) Activity Assay Kit
AK0197/AK0196	β-xylosidase Activity Assay Kit