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Filter paper enzyme (FPA) Activity Assay Kit

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

Operation Equipment: Spectrophotometer

Cat No: AK0191

Size:50T/24S

Components:

Reagent I: 35mL×1. Storage at 4℃ .

Reagent II: 60mL×1. Storage at 4℃ .

filter paper: 50mg×50 filter papers, moisture-proof storage at room temperature

Standard: Powder×1, 10 mg anhydrous glucose. Add 1 mL of distilled water to dissolve to prepare a 10 mg/mL glucose standard solution for use, and store at 4℃ for one week.

Product Description:

Cellulase is a multi-component enzyme system produced by microorganisms, which can hydrolyze cellulose β- 1,4 glucosidic bonds to produce glucose. Studying the activity of filter paper enzymes is of great significance to the study of cellulase.

The reducing sugar produced by the hydrolysis of the filter paper enzyme can generate red-brown amino compounds with 3,5-dinitrosalicylic acid. It has the maximum light absorption at 540nm. The color of the reaction solution is proportional to the amount of reducing sugar within a certain range. The filter paper enzyme activity can be determined.

Reagents and Equipment Required but Not Provided:

Spectrophotometer, water bath, balance, centrifuge, adjustable transferpettor, 1 mL glass cuvette, mortar/homogenizer, ultrasonic crusher, ice and distilled water.

Procedure:

I. Enzyme extraction

1. Tissue

According to the tissue mass (g): the volume of distilled water (mL) is 1:5~10 to extract. It is recommended to add 1 mL of distilled water to 0.1 g of tissue, and fully homogenize on ice bath. Centrifuge at 12000rpm for 10 minutes at 4℃ to remove insoluble materials, and take the supernatant on ice before testing.

2. Bacteria or cells

According to the bacteria or cells (10^4): the volume of distilled water (mL) is 500~1000:1 to extract. It is recommended to add 1 mL of water to 5 million of bacteria or cells. Use ultrasonication to split bacteria and cells (placed on ice, ultrasonic power 300W, working time 3s, interval 7s, total time 3 min). Centrifuge at 12000rpm for 10 minutes at 4℃ to remove insoluble materials and take the supernatant on ice before

testing.

3. Culture medium or other liquid: Detect directly.

II. Detection

1) Preheat spectrophotometer for 30 minutes, adjust the wavelength to 540 nm, set zero with distilled water.

2) Standard: Dilute the 10 mg/mL standard solution to 0.8, 0.6, 0.5, 0.4, 0.3, 0.2 mg/mL with distilled water.

3) Take 200 μL of the crude enzyme and boil it for 10 minutes to deactivates it and as the contrast tube.

4) Add the following reagents in 1.5 mL EP tubes:

Reagent (μL)	Contrast tube (C)	Test tube (T)	Standard tube (S)	Blank tube (B)
Deactivated crude enzyme	200	-	-	-
Crude enzyme	-	200	-	-
Put a rolled filter paper strip **at the bottom** into each EP tube as substrate.				
Distilled water	-	-		200
Standard solution	-	-	200	
Reagent I	500	500	500	500
Mix thoroughly and incubate accurately at 50℃ water bath for 30minutes.				
Reagent II	800	800	800	800
Mix thoroughly and place the tubes in a boiling water bath for 5 minutes (cover tightly to prevent moisture loss) and rapid cooling by ice bath. Detect the absorbance at 540 nm, record as A_C, A_T, A_S and A_B respectively. $\Delta A_T=(A_T-A_C)$, $\Delta A_S=(A_S-A_B)$. A contrast tube is required for each test tube, and the standard curve need only be tested once or twice.				

II. Calculation:

1. Standard curve

The concentration of standard solution as x-axis, ΔA_S as y-axis, obtain the equation y=kx+b. Take ΔA_T to the equation to acquire x (mg/mL) value.

2. **Calculation**

1) Tissue protein concentration

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes that the generation of 1 mg of glucose every mg of protein in the reaction system per minute at 40℃ .

FPA Activity (U/mg prot)=x×Ve÷ （Ve×Cpr） ÷T =0.0333x÷ Cpr

2) Tissue weight

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes that the generation of 1 mg of glucose in the reaction every gram of tissue system per minute at 40℃ .

FPA Activity (U/g weight) = x×Ve÷ W÷T =0.0333x÷ W

3) Liquid

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes that the generation of 1 mg of glucose in the reaction system every milliliter of liquid sample per minute at 40℃ .

FPA Activity (U/mL)=x× Vs÷Vs÷T=0.0333x

4) Bacteria or cultured cells

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes that the generation of 1 mg of glucose in the reaction system every 10^4 cells or bacteria per minute at 40℃ .

FPA Activity (U/10^4 cell)=x× Ve÷ cell amount÷ T= 0.0333x÷ cell amount

Vs: Sample volume (mL), 0.2 mL;

Ve: Extract solution volume, 1 mL;

Cpr: Supernatant sample protein concentration (mg/mL);

T: Reaction time (min), 30 minutes;

W: Sample weight, g;

Cell amount: 10 thousand as unit.

Note:

1. Remove the filter paper strip with clean tweezers, roll the filter paper strip into a small roll with gloves and place it **in the bottom** of the Ep tube.

2. When A or ΔA exceeds 1.2, it is recommended to measure the sample after diluting it with distilled water. Multiply the dilution factor in the calculation formula.

3. At the end of color development, when sucking the test solution, pay attention not to touch the filter paper with the gun head, so as not to bring in hair and affect the test results.

Experimental example:

1. Take 0. 1g of umbrella part of pleurotus ostreatus, add 1 mL of distilled water, homogenize in ice bath, centrifuge at 4℃ and 12000 rpm for 10 min, and place the supernatant on ice for testing. Then, measure by 96 well plate according to the determination steps, calculate $\Delta A= A_T-A_C=0.668-0.425=0.243$, bring in the measured standard curve y = 1.6716x - 0.2189, calculate x = 0.2763.

FPA activity (U/g mass) = $x \times V_E \div W \div T = 0.0333x \div W = 0.092$ U/g mass.

Related Products :

AK0229/AK0227	Plant Tissue Fructose Content Assay Kit
AK0211/AK0210	Cellulase(CL) Activity Assay Kit
AK0295/AK0294	Trehalose Content Assay Kit
AK0218/AK0217	Trehalase Activity Assay Kit
AK0216/AK0214	Sorbitol Content Assay Kit