Filter Paper Enzyme (FPA) Activity Assay Kit

Note: It is necessary to predict 2-3 large difference samples before the formal determination.

Operation Equipment: Spectrophotometer/ Microplate reader

Cat No: BC2655

Size:100T/48S

Components:

Reagent I: Liquid 35mL×1. Store at 2-8℃.

Reagent II: Liquid 60mL×1. Store at 2-8℃.

Filter paper: 25mg×100 filter papers, moisture-proof store 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 2-8℃ for two weeks.

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/ microplate reader, water bath, balance, centrifuge, adjustable transferpettor, micro glass cuvette/ 96 well flat-bottom plate, mortar/ homogenizer/cell ultrasonic crusher, ice and distilled water, EP tube (2mL).

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 number (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 5 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 (If the solution is turbid, take the supernatant after centrifugation for measurement).

II. Detection

1) Preheat spectrophotometer/microplate reader for 30 minutes, adjust the wavelength to 540 nm and set spectrophotometer counter to zero with distilled water.
2) Standard working solution: Dilute the 10 mg/mL standard solution to 1, 0.8, 0.6, 0.5, 0.4, 0.3, 0.2 mg/mL with distilled water.
3) Take 100 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 2 mL EP tubes:

Reagent (μL)	Contrast tube (C)	Test tube (T)	Standard tube (S)	Blank tube (B)
Deactivated crude enzyme	100	-	-	-
Crude enzyme	-	100	-	-
Put a rolled filter paper strip **at the bottom** into each EP tube as substrate.			-	-
Distilled water	-	-	-	100
Standard solution	-	-	100	-
Reagent I	250	250	250	250
Mix well and incubate accurately at 50℃ water bath for 30minutes.			-	-
Reagent II	400	400	400	400
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 and blank tube need only be tested once or twice.				

III. Calculation:

1. Standard curve

According to the concentration of the standard tube (x, mg/mL) and the absorbance ΔA_S (y, ΔA_S), establish a standard curve. Based on the standard curve, bring the ΔA_T (y, ΔA_T) into the formula to calculate the sample concentration (x, mg/mL).

2. Calculation

1) Tissue protein concentration

Unit Definition: One unit of enzymatic activity is defined as 1 mg of glucose produced per minute per milligram of protein by breaking down the filter paper.

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

2) Tissue weight

Unit Definition: One unit of enzymatic activity is defined as 1 mg of glucose produced per minute per gram of tissue by breaking down the filter paper.

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

3) Bacteria or cultured cells number

Unit Definition: One unit of enzymatic activity is defined as 1 mg of glucose produced per minute per 10^4 cells by breaking down the filter paper.

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

4) Liquid volume

Unit Definition: One unit of enzymatic activity is defined as 1 mg of glucose produced per minute per milliliter by breaking down the filter paper.

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

Vs: Sample volume, 0.1 mL;

Ve: Extract solution volume, 1 mL;

Cpr: Supernatant sample protein concentration, mg/mL;

T: Reaction time, 30 minutes;

W: Sample weight, g;

N: Bacteria or cultured cells number, 10^4 per 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, 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 $\Delta A= A_T-A_C=$ 0.744-0.536 = 0.208, bring in the measured standard curve y = 1.0495x-0.1471, calculate x = 0.3384, calculate the enzyme activity according to the sample weight:

   FPA activity (U/g mass) = x × V_E ÷ W ÷ T = 0.0333x ÷ W = 0.113 U/g mass.

Related Products：

BC2450/BC2455	Plant Tissue Fructose Content Assay Kit
BC2540/BC2545	Cellulase(CL) Activity Assay Kit
BC0330/BC0335	Trehalose Content Assay Kit
BC2510/BC2515	Trehalase Activity Assay Kit
BC2520/BC2525	Sorbitol Content Assay Kit