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Saccharifying enzyme Activity Assay Kit

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

Operation Equipment: Spectrophotometer

Cat No: AK0187

Size:50T/24S

Components:

Extract solution: Liquid 110 mL×1. Storage at 4℃ .

Reagent I: Powder×1. Storage at 4℃ . Add 40 mL of Extract solution before use. Mix thoroughly, then place in a boiling water bath for 10 minutes to make it fully dissolved.

Reagent II: Liquid 35 mL×1. Storage at 4℃ and protect from light.

Standard: Powder×1. Storage at 4℃ . Dissolve the standard with 1 mL of Extract solution to generate a 10 mg/mL glucose solution standard, store at 4℃ and use within one week.

Product Description:

Saccharifying enzyme, glucoamylase (EC3.2. 1.3) also known as γ- amylase, which is an extracellular enzyme that is secreted by a series of microorganisms and has exonuclease activity. The main role of Saccharifying enzyme is to hydrolyze α- 1,4 glycosidic bonds in sequence from non-reducing ends on the carbon chains such as starch, dextrin, glycogen, etc., and cut off each glucose unit. For amylopectin, when it encounters a branch point, it can also hydrolyze α- 1,6 glycosidic bonds, thereby hydrolyzing all amylopectin to glucose. It is one of the important industrial enzyme preparations in China, and is widely used in alcohol, liquor, antibiotics, amino acids, organic acids, glycerol, starch sugar and other industries.

Saccharifying enzyme converts soluble starch to glucose. Under the alkaline condition, 3.5-Dinitrosalicylic acid is reduced to brown-red amino compound by co-heating with glucose. The brown-red amino compound has a maximum absorption peak at 540 nm and the absorbance ratio is in direct proportion to the contents of glucose. In this kit, the saccharifying enzyme activity is quantified by measuring the color development at 540 nm.

Reagents and Equipment Required but Not Provided:

Spectrophotometer, water bath, balance, desk 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 the Extract solution (mL) is 1:5~10 to Extract solution. It is recommended to add 1 mL of Extract solution to 0.1 g of tissue, and fully homogenized on ice bath. Centrifuge at 10000×g 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 the Extract solution (mL) is 500~1000:1 to extract. It is recommended to add 1 mL of Extract solution to 5 million of bacteria or cells. Use ultrasonication to splitting bacteria and cells (placed on ice, ultrasonic power 300W, working time 3s, interval 7s, total time 3 min). Centrifuge at 10000×g 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 1.5, 1, 0.8, 0.4, 0.2, 0.1 mg/mL with distilled water.

3) Take 50 μL of the crude enzyme and boil it for 5 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	50	-	-	-
Crude enzyme	-	50	-	-
Distilled water	-	-	-	50
Standard solution	-	-	50	-
Reagent I	500	500	500	500
Mix thoroughly and incubate accurately at 40℃ water bath for 20 minutes, then place the tubes in a boiling water bath for 5 minute (cover tightly to prevent moisture loss) and rapid cooling by ice bath. Centrifuge at 10000×g for 10 minutes at room temperature to remove insoluble materials and take the supernatant				
Supernatant	500	500	500	500
Reagent II	500	500	500	500
Mix thoroughly and place the tubes in a boiling water bath for 5 minute (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. $\Delta A_T=(A_T-A_C)$, $\Delta A_S=(A_S-A_B)$.				

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 enzymes catalyzes the generation of 1 mg of glucose in the reaction system per hour at 40℃ every mg protein.

Saccharifying enzyme Activity(U/mg prot)=x×Ve÷(Ve×Cpr)÷T =3x÷ Cpr

2) Tissue weight

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

Saccharifying enzyme Activity(U/g weight)= x×Ve÷ W÷T =3x÷ W

3) Liquid

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

Saccharifying enzyme Activity(U/mL)=x× Vs÷Vs÷T=3x

4) Bacteria or cultured cells

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

Saccharifying enzyme Activity(U/10^4 cell)=x×Ve÷500÷T= 0.006x

Vs: Sample volume (mL), 50 μL=0.05 mL;

Ve: Extract solution volume, 1 mL;

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

T: Reaction time (min), 20 minutes = 0.333 hour;

W: Sample weight, g;

500: 5 million cells or bacteria.

Note:

1. Take two or three different samples for prediction before test. If the absorption value is higher, the sample can be determined after being appropriately diluted. When calculation, multiply the calculation formula by the corresponding dilution factor.

Experimental example:

1. 0. 1g magnolia leaves is homogenized by adding 1 mL of Extract solution in ice bath, then centrifuged at 4℃ and 10000g for 10 min. The supernatant is put on ice, and then the operation is carried out according to the determination steps. $\Delta A_T = A_T - A_C$ =0.738-0.584=0. 154 is measured, and the standard curve y=0.7372x+0.0175, and x = 0.18516 is calculated.

Glucoamylase activity (U/g mass) = 3x ÷ W = 5.55 U/g mass.

2. Take 0. 1g liver, add 1 mL of Extract solution, homogenize in ice bath, centrifuge at 4℃ and 10000g for 10min, take the supernatant and put it on ice, then operate according to the determination steps, measure and calculate $\Delta A = A_T - A_C$ = 1.420- 1.282=0. 138, bring in the standard curve y=0.7372x+0.0175, calculate: x = 0. 163, calculate the enzyme activity according to the sample mass.

Glucoamylase activity (U/g mass) = 3x ÷ W = 4.89 U/g mass.

3. The rabbit serum is taken and operated according to the determination steps. The calculation of ΔA measured: $\Delta A = A_T - A_C$=1.447-0.753=0.694. The standard curve y=0.7372x+0.0175, and x=0.918 is calculated.

Glucoamylase activity (U/mL) = 3x = 2.754 U/mL.

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