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α-amylase (α-AL) Activity Assay Kit(Iodine-starch colorimetry)

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

Detection instrument: Spectrophotometer/microplate reader

Cat No: AK0046

Size: 100T/48S

Components:

Reagent I: Powder×1. Store at 4℃ . Add 12.5 mL of Reagent III when the solution will be used. The solution is placed in water at room temperature. Heat to boil, stir continuously until the powder dissolves completely.

Reagent II A: Powder×1. Store at 4℃ .

Reagent II B: Powder×1. Store at 4℃ .

Reagent II: Pour Reagent II A to Reagent II B, make up to 10 mL with distilled water. Store at 4 ℃ in the dark for one month.

Reagent III: 40 mL×1. Store at 4℃.

Standard: Powder×1, 10 mg of starch. Add 10 mL of Reagent III to form 1 mg/mL starch standard solution when the solution will be used. Dissolve by shaking in a boiling water bath to prepare a 1 mg/mL starch standard solution.

Product Description:

Amylase including α-amylase and β-amylase. α-amylase (α-AL, EC 3.2.1.1) randomly catalyze the hydrolysis of α-1,4-glycosidic bonds in starch to produce reducing sugars such as glucose, maltose, maltotriose, dextrin, etc. At the same time, the viscosity of starch is reduced, so it is also called liquifying enzyme.

α-Amylase catalyzes the hydrolysis of α-1,4 glycosidic bonds in starch molecules to produce glucose, maltose, dextrin, etc. Iodine can be combined with starch that is not hydrolyzed by amylase to form a complex with a characteristic absorption peak at 570 nm. The depth can calculate the unit of amylase activity. α-amylase is acid-resistant and β-amylase is heat-resistant. According to the above characteristics, the activity of another amylase can be measured by passivating one of them.

Required material:

Spectrophotometer, thermostat water bath, desktop centrifuge, transferpettor, micro glass cuvette/96 well plate, mortar, distilled water.

Procedure:

I. Sample extraction:

1. Tissue:

It is suggested that when weigh about 0.1 g of sample, add 1 mL of distilled water. After homogenize,

place the extract at room temperature and extract for 15 minutes. Shake once every 5 minutes to fully extracted. Centrifuge at 6000 ×g for 10 minutes at room temperature, the supernatant is the amylase stock solution for test.

2. Liquid sample:Direct detection.

II. Detection

1. Preheat spectrophotometer/microplate reader for 30 minutes, adjust wavelength to 570 nm, set zero with distilled water.

2. Dilute the 1 mg/mL starch standard solution with distilled water to 0.4、0.2、0.1、0.05、0.025、0.0125、0.00625mg/mL.

3. Add each reagent in turn according to the operation table

Reagent Name (μL)	Test tube (T)	Contrast tube (C)	Blank tube (B)	Standard tube (S_1)	Standard blank tube (S_0)
α-amylase stock solution	100	100	-	-	-
Distilled water	-		100	-	100
Standard solution	-	-		100	-
Incubate in 70℃ water bath for 15 minutes, cooling.					
Reagent I	100	-	100	-	-
Distilled water		100	-	100	100
Incubate in 40℃ thermostat water bath for 10 minutes.					
Reagent II	50	50	50	50	50

Mix well, measure the absorbance at 570 nm, recorded as A_T, A_C, A_B, A_{S1}, and A_{S0} respectively from left to right. $\Delta A\alpha = A_B-(A_T-A_C)$, $\Delta A_{Standard} = A_{S1}-A_{S0}$.

III. Calculation:

1. Create standard curve

Using the concentration of standard solution as x axis and $\Delta A_{Standard}$ as y axis create standard curve, obtain equation y=kx+b. Put ΔAα into the equation and obtain the x1(mg/mL), Put ΔA_{Total} into the equation and obtain the x2(mg/mL).

2. Calculation of α-amylase activity

(1) Calculation by fresh weight of sample

Definition of unit: One unit is defined as an enzyme activity that per gram of tissue catalyze the hydrolyze of 1 mg of starch per minute.

α-amylase activity (U/g fresh weight)=$x_1 \times V_S \div (W \times V_S \div V_{ST}) \div T = 0.1 \times x_1 \div W$

(2) Calculation according to protein content

Definition of unit: One unit is defined as an enzyme activity that per milligram of tissue protein catalyze the hydrolyze of 1 mg of starch per minute.

α-amylase activity (U/mg prot) = $x_1 \times V_S \div (V_S \times Cpr) \div T = 0.1 \times x_1 \div Cpr$

(3) Calculation according to liquid sample

Definition of unit: One unit is defined as an enzyme activity that per milligram of tissue protein catalyze

the hydrolyze of 1 mg of starch per minute.

α-amylase activity(U/mL)= $x_1 \times V_S \div V_S \div T = 0.1 \times x_1$

V_S : The volume of sample added to reaction system, 0.1 mL;

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

Cpr: Sample protein concentration, mg/mL;

T: Reaction time, 10 minutes;

W: Sample weight, g.

Note:

When the measured absorbance value is greater than 1.5 or ΔA is greater than 0.8, the sample can be appropriately diluted for determination.

Experimental Examples:

1. Take about 0.1g of quinoa leaves and add 1mL of distilled water to homogenize. After homogenization, place it at room temperature for 15min, shake every 5min to make it fully extracted; centrifuge at 6000g at room temperature for 10min, absorb the supernatant, and then follow In the determination step operation, the measured calculation ΔAt=Ab-(At-Ac) =1.065-（0.849-0.220）=0.436, bring into the standard curve y=2.628x-0.0051, calculate x=0.168, according to the sample quality Calculate enzyme activity:

α-amylase activity (U/g mass)=0.1×x÷W=0.168 U/g mass.

Related Products:

AK0045/AK0044 β-Amylase Assay Kit(Iodine-starch colorimetry)