β-amylase Activity Assay Kit (Iodine-Starch Colorimetry)

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

Operation Equipment: Spectrophotometer/ Microplate reader

Cat No: BC4585

Size: 100T/48S

Components:

Reagent I：Powder ×2. Storage at 4℃. Before use, take one bottle and add 7.5 mL of Reagent III, put it in normal temperature water and heat it to boil. During the process, stir the powder to dissolve, and keep the reagent in 4℃ for one week;

Reagent IIA: Powder ×1. Storage at 4℃.

Reagent IIB: Powder ×1. Storage at 4℃. Pour Reagent IIA into Reagent IIB, fix the volume to 12 mL with distilled water, and store in dark at 4℃ for one month;

Reagent III: Liquid 40 mL×1. Storage at 4℃.

Standard solution: Powder ×1. Storage at 4℃. 10 mg of starch standard. Before use, add 10 mL of Reagent III, put it in boiling water bath to shake and dissolve, and prepare 1 mg/mL starch standard solution.

Product Description:

Amylase is responsible for the hydrolysis of starch, mainly including α-amylase and β-amylase. β-amylase (EC 3.2.1.2) cleaves α-1,4 glycosidic bonds from the non reducing end of starch to form reducing sugars such as glucose, maltose, maltotriose and dextrin.

Iodine can combine with starch which is not hydrolyzed by amylase to form a complex with characteristic absorption peak at 570 nm. The activity unit of amylase can be calculated from the depth of the complex. α-AL is acid resistant and β-Al is heat resistant. According to the above characteristics, passivating one of them can measure the other activity.

Reagents and Equipment Required but Not Provided:

Spectrophotometer/ Microplate reader, constant temperature water bath, table centrifuge, transferpettor, micro glass cuvette/96 well plate, mortar/homogenizer, distilled water.

Procedure:

I. Sample preparation (the sample size can be adjusted appropriately, and the specific proportion can be referred to the literature):

1. Tissue: weigh about 0.1 g of sample, add 1 mL of distilled water to homogenize; after homogenization, place the sample at room temperature for 15 min, and shake it every 5 min to make it fully extracted; centrifuged at room temperature, 6000g for 10min, and absorb the supernatant to be amylase stock solution.

2. Liquid: direct detection.

II. Determination procedure:

1. Preheat the Spectrophotometer for 30 minutes, adjust the wavelength to 570 nm, set zero with distilled water.
2. The starch standard solution is diluted with distilled water to 0.4、0.2、0.1、0.05、0.025、0.0125、0.00625 mg/mL.
3. Add reagents with the following list:

Reagent（μL）	Determination of α-amylase activity		Determination of total-amylase activity		Blank tube 5（A5）	Determination of standard curve	
	Test tube 1（A1）	Control tube 2（A2）	Test tube 3（A3）	Control tube 4（A4）		Standard tube 6（A6）	Standard blank tube 7（A7）
Sample	100	100	-	-	-	-	-
Distilled water	-	-	-	-	100	-	100
Standard solution	-	-	-	-	-	100	-
Water bath at 70℃ for about 15 minutes, cooling							
Sample	-	-	100	100	-	-	-
Reagent I	100	-	100	-	100	-	-
Distilled water	-	100	-	100	-	100	100
Keep the temperature at 40℃ for 5 min							
Reagent II	50	50	50	50	50	50	50

After mixing, determine the absorbance at 570 nm in 1 mL glass cuvette, and record it as A1, A2, A3, A4, A5 and A6 from left to right. Calculate $\Delta A\alpha$ = A5 - (A1-A2), ΔA_T= A5 - (A3-A4), ΔA_S=A6-A7

III. Calculation:

1. Drawing of standard curve: draw the standard curve with ΔA_S as y axis, and the standard solution concentration as x axis, and get the standard equation y=kx+b, and bring the $\Delta A\alpha$ into the equation to get x_1(μmol/mL), bring the ΔA_T into the equation to get x_2(μmol/mL)

2. Calculation of α-amylase activity

(1) Calculated by sample mass

Unit definition: One unit of enzyme is defined as the amount of enzyme catalyzes the consume of 1 mg of starch per minute every gram tissue.

α- amylase activity (U/g mass) = $x_1 \times V_S \div (W \times V_S \div V_{ST}) \div T = 0.2 \times x_1 \div W$

(2) Calculated according to protein concentration

Unit definition: One unit of enzyme is defined as the amount of enzyme catalyzes the consume of 1 mg of starch per minute every gram tissue protein.

α-amylase activity (U/mg prot) = $x_1 \times V_S \div (V_S \times Cpr) \div T = 0.2 \times x_1 \div Cpr$

(3) Calculated according to volume of liquid:

Unit definition: One unit of enzyme is defined as the amount of enzyme catalyzes the consume of 1 mg of starch per minute every milliliter liquid.

α-amylase activity (U/mL) = $x_1 \times V_S \div V_S \div T = 0.2 \times x_1$

V_S: sample volume added into the reaction system, 0.1 mL; V_{ST}: sample total volume, 1 mL; Cpr: sample protein concentration, mg/mL; W: sample mass, g; T: reaction time, 5 min.

3. Calculation of total-amylase activity

(1) Calculated by sample mass

Unit definition: One unit of enzyme is defined as the amount of enzyme catalyzes the consume of 1 mg of starch per minute every gram tissue.

Total - amylase activity (U/g mass) = $x_2 \times V_S \div (W \times V_S \div V_{ST}) \div T = 0.2 \times x_2 \div W$

(2) Calculated according to protein concentration

Unit definition: One unit of enzyme is defined as the amount of enzyme catalyzes the consume of 1 mg of starch per minute every gram tissue protein.

Total -amylase activity (U/mg prot) = $x_2 \times V_S \div (V_S \times Cpr) \div T = 0.2 \times x_2 \div Cpr$

(3) Calculated according to volume of liquid:

Unit definition: One unit of enzyme is defined as the amount of enzyme catalyzes the consume of 1 mg of starch per minute every milliliter liquid.

Total -amylase activity (U/mL) = $x_2 \times V_S \div VS \div T = 0.2 \times x_2$

V_S: sample volume added into the reaction system, 0.1 mL; V_{ST}: sample total volume, 1 mL; Cpr: sample protein concentration, mg/mL; W: sample mass, g; T: reaction time, 5 min.

4. Calculation of β-amylase activity

(1) Calculated by sample mass

Unit definition: One unit of enzyme is defined as the amount of enzyme catalyzes the consume of 1 mg of starch per minute every gram tissue.

β - amylase activity (U/g mass) = total -amylase activity -α-amylase activity = $0.2 \times x_2 \div W - 0.2 \times x_1 \div W$

(2) Calculated according to protein concentration

Unit definition: One unit of enzyme is defined as the amount of enzyme catalyzes the consume of 1 mg of starch per minute every gram tissue protein.

β -amylase activity (U/mg prot) = total -amylase activity -α-amylase activity = $0.2 \times x_2 \div Cpr - 0.2 \times x_1 \div Cpr$

(3) Calculated according to volume of liquid:

Unit definition: One unit of enzyme is defined as the amount of enzyme catalyzes the consume of 1 mg of starch per minute every milliliter liquid.

β -amylase activity (U/mL) = total -amylase activity -α-amylase activity = $0.2 \times x_2 - 0.2 \times x_1$

Note:

When the absorbance value is greater than 1.5 or ΔA is greater than 0.8, the sample can be determined after appropriate dilution.

Experimental example:

1. Take about 0.1 g of Chenopodium album leaves for sample treatment, take the supernatant and operate according to the determination steps, calculate $\Delta A\alpha = A5 - (A1 - A2) = 1.065 - (0.849 - 0.220) = 0.436$, $\Delta A_T = A5 - (A3 - A4) = 1.065 - (0.677 - 0.197) = 0.585$ with 96 well plate, carry in the standard curve y=2.628x-

0.0051, calculate x_1=0.1678, x_2 = 0.2245, calculate the enzyme activity according to the sample mass β - amylase activity (U/g mass) =0.2×x_2÷W-0.2×x_1÷W =0.2×0.2245÷0.1-0.2×0.1678÷0.1=0.1134 U/g mass.

Related products:

BC4570 / BC4575 α-amylase (α - AL) Activity Assay Kit (Iodine-Starch Colorimetry)