α-Amylase(α-AL) Activity Assay Kit (Iodine-starch colorimetric method)

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

Detection instrument: Spectrophotometer/microplate reader

Cat No: BC4575

Size: 100T/48S

Product Composition: Before use, please carefully check whether the volume of the reagent is consistent with the volume in the bottle. If you have any questions, please contact Solarbio staff in time.

Reagent name	Size	Preservation Condition
Reagent I	Powder×1	2-8℃
Reagent II	Liquid 10 mL×1	2-8℃
Reagent III	Liquid 30 mL×1	2-8℃
Standard	Powder×1	2-8℃

Solution Preparation:

1. Reagent I: add 6.25 mL of reagent III before use, put it in water at room temperature and heat it to boiling, stir the powder constantly until it is dissolved during the period, and store the inexhaustible reagent at 2-8°C for 8 weeks;

2. Standard: 10 mg of starch standard. Add 10 mL of reagent III before use, put it into boiling water bath and shake to dissolve, formulate into 1 mg/mL starch standard solution and keep it at 2-8℃ for four weeks.

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 liquefied enzyme.

α-amylase catalyzes the hydrolysis of the α-1,4 glycosidic bond in the starch molecule to produce glucose, maltose, and dextrin, etc. Iodine can be combined with unhydrolyzed starch to form a complex with a characteristic absorption peak at 570 nm, the depth of which can be used to calculate the viability unit of the enzyme. α-AL is heat-resistant, but β-amylase can be passivated for 15 min at 70°C. Therefore, after passivation of crude enzyme at 70°C for 15 min, only α-AL is able to catalyze the hydrolysis of starch. Therefore, after the crude enzyme solution is passivated at 70°C for 15 min, only α-AL can catalyze starch hydrolysis.

Required material:

Spectrophotometer/microplate reader, thermostat water bath, desk centrifuge, adjustable pipette, micro glass cuvette/96 well flat-bottom plate, mortar/homogenizer, 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, extract at room temperature for 15 minutes. Shake once every 5 minutes to fully extracted. Centrifuge at 6000×g for 10 minutes at room temperature. Take supernatant on ice before testing.

（2）Serum (plasma) : direct detection

II. Determination procedure:

1 Preheat the spectrophotometer/microplate reader for 30 minutes, adjust wavelength to 540 nm, and set spectrophotometer counter to zero with distilled water.

2 Standard working solution: dilute the glucose standard solution 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)	Test tube (T)	Control tube(C)	Blank tube (B)	Standard tube(S)	Standard -Blank tube (B')
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 5 minutes.					
Reagent II	50	50	50	50	50

Mix well and take 200μL of the reaction solution to micro glass cuvette or 96-well flat-bottom plate, measure the absorbance at 570 nm. Record as A_T, A_C, A_B, A_S, $A_{B'}$, and calculate $\triangle A_S = A_S - A_{B'}$, $\triangle A_T = A_B - (A_T - A_C)$. Each test tube should be provided with one contrast tube. Standard curve and blank tube only need to be measured once or twice.

III. Calculation:

1 Create standard curve

Taking the concentration of each standard solution as the x-axis and its corresponding ΔA_S as the y-axis, draw a standard curve to get the standard equation $y = kx + b$, and bring ΔA_T into the equation to get x (mg/mL).

2 Enzyme activity calculation:

1) Calculated by sample weight

Unit definition: Consumption of 1 mg of starch per minute per g of tissue is defined as 1 unit of enzyme activity.

α-amylase (U/g weight) =x×Vs÷(W×Vs÷Ve) ÷T= 0.1×x÷W

2) Calculated by protein concentration:

Unit definition: Consumption of 1 mg of starch per minute per mg of protein is defined as 1 unit of enzyme activity.

α-amylase (U/mg prot) =x×Vs÷(Cpr×Vs) ÷T=0.1×x÷Cpr

3) Calculated by Liquid volume

Unit definition: Consumption of 1 mg of starch per minute per mL of liquid sample is defined as 1 unit of enzyme activity.

α -amylase (U/mL) =x×Vs÷Vs÷T= 0.1×x

Ve: Extract solution volume,1 mL;
Cpr: Sample protein concentration, mg/mL;
T: Reaction time, 10 minutes;
W: Sample weight, g;
Vs: Volume of sample added to the reaction system, 0.1mL.

Note:

If the absorbance value is greater than 1.5 or ΔA is greater than 0.8, the sample can be diluted appropriately and measured.

References:

[1] Xiao Z, Storms R, Tsang A. A quantitative starch-iodine method for measuring alpha-amylase and glucoamylase activities[J]. Analytical Biochemistry, 2006, 351(1): 146-148.

[2] Gaenssle ALO, van der Maarel MJEC, Jurak E. Reliability factor for identification of amylolytic enzyme activity in the optimized starch-iodine assay[J]. Analytical Biochemistry, 2020, 597:113696.

Related Products:

BC0430/BC0435	ADPG Pyrophosphorylase(AGP) Activity Assay Kit
BC1850/BC1855	Soluble Starch Synthase (SSS) Activity Assay Kit
BC3290/BC3295	Bound Station amylosynthease Activity Assay Kit