α-Amylase(α-AL) Activity Assay Kit

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

Detection instrument: Spectrophotometer/microplate reader

Cat No: BC0615

Size: 100T/48S

Components:

Reagent I: Liquid 20 mL×1. Store at room temperature. If yellow crystal is precipitated, heated moderately to dissolve before use.

Reagent II: Liquid 5 mL×2. Store at 4℃. Each Reagent II is added to each Reagent III. The solution is placed in room temperature water, heat with frequent agitation and boil to completely dissolve the powder. It could be stored at 4℃ for four weeks.

Reagent III: Powder×2. Store at 4℃.

Standard: Powder×1. Store at 4℃. 10 mg anhydrous glucose. Add 1 mL of distilled water to form 10 mg/mL glucose standard solution when the solution will be used. It could be stored at 4℃ for two 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.

Starch hydrolase catalyzes the hydrolysis of starch to produce reducing sugar. 3,5-dinitrosalicylic acid is reduced to brown red substance by the reducing sugar, and the brown red substance has an absorption peak at 540 nm. The activity of amylase is calculated by measuring the increasing rate of absorbance at 540 nm. α-AL is thermostable, but β-AL could be passivated at 70℃ for 15 minutes. Therefore, only α-AL could catalyze starch hydrolysis when the crude enzyme solution is passivated at 70℃ for 15 minutes.

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:

It is suggested that when weigh about 0.1 g of sample, add 0.8 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 the supernatant and add distilled water to 10 mL, shake well, that is the original amylase solution.

II. Determination procedure:

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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 0.5, 0.25, 0.125, 0.0625, 0.03125, 0.015625, 0.0078 mg/mL.

3 250 μL of sample is used as control tube in boiling water bath for 5 min.

4 Add reagents with the following list:

Reagent (μL)	Control tube(C)	Test tube (T)	Standard tube(S)	Blank tube (B)
α-amylase stock solution	75(Boiling sample)	75	-	-
Distilled water	-	-	-	75
Standard Solution	-	-	75	-
Incubate in 70℃ water bath for 15 minutes, cooling.				
Reagent II	-	75	-	-
Incubate in 40℃ thermostat water bath for 5 minutes.				
Reagent I	150	150	150	150
Reagent II	75	-	75	75

Mix well, boiling water bath for 10 minutes, then take 200μL of the reaction solution to micro glass cuvette or 96-well flat-bottom plate, measure the absorbance at 540 nm. Record as A_T, A_C, A_S, A_B, and calculate $\triangle A_S = A_S - A_B$, $\triangle A_T = A_T - A_C$. 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: One unit of enzyme activity is defined as the amount of enzyme catalyzes the production of 1 mg of reducing sugar per minutes every gram of tissue.

α-amylase (U/min/g fresh weight) =x×Vs÷(W×Vs÷Ve) ÷T= 2×x÷W

2) Calculated by protein concentration:

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the production of 1 mg of reducing sugar per minutes every milligram of protein.

α-amylase (U/min/mg prot) =x×Vs÷(Cpr×Vs) ÷T= 0.2×x÷Cpr

Vs: Sample volume in reaction system, 0.075 mL;

Ve: Extract solution volume,10 mL;

Cpr: Sample protein concentration, mg/mL;

T: Reaction time, 5 minutes;

W: Sample weight, g.

Note:

If the absorbance value is greater than 1.5, the sample should be diluted properly and then determined. If the absorbance value is too small, the original amylase solution or diluted amylase solution can be concentrated.

Recent Products References:

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[5] Zheng X, Xiao H, Chen J, Zhu J, Fu Y, Ouyang S, Chen Y, Chen D, Su J, Xue T. Metabolome and Whole-Transcriptome Analyses Reveal the Molecular Mechanisms Underlying Hypoglycemic Nutrient Metabolites Biosynthesis in Cyclocarya paliurus Leaves During Different Harvest Stages. Front Nutr. 2022 Feb 28;9:851569. doi: 10.3389/fnut.2022.851569. PMID: 35295916; PMCID: PMC8919051.

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[2] Liu Hehong, Wan Jinquan, Ma Yongwen. et al. Study on Determination of Amylase Activity in Activated Sludge with DNS Spectrophotometry[J]. Journal of Anhui Agricultural Sciences, 2008, 36(33): 14369-14371.

Related Products:

BC0430/BC0435 ADPG Pyrophosphorylase(AGP) Activity Assay Kit

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