α-Amlase (α-AL) Activity Assay Kit

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

Detection instrument: Spectrophotometer

Cat No: BC0610

Size: 50T/24S

Components:

Reagent I: Liquid 40 mL×1. Store at room temperature. If yellow crystal is precipitated, heated moderately to dissolve before use.

Reagent II: Liquid 10 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, thermostat water bath, desk centrifuge, adjustable pipette, 1 mL glass cuvette, mortar/homogenizer, distilled water.

Procedure:

I. Sample Extraction:

It is suggested that 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:

1 Preheat the spectrophotometer for 30 minutes, adjust wavelength to 540 nm, set zero with distilled water.

2 Standard working solution: dilute the glucose standard solution with distilled water to 0.2、0.1、0.05、0.025、0.0125、0.00625 mg/mL.

3 Take 250 μL of sample and take a boiling bath for 5 minutes which use as control tube.

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	250(Boiling sample)	250	-	-
Distilled water	-	-	-	250
Standard Solution	-	-	250	-
Incubate in 70℃ water bath for 15 minutes, cooling.				
Reagent II	-	250	-	-
Incubate in 40℃ thermostat water bath for 5 minutes.				
Reagent I	500	500	500	500
Reagent II	250	-	250	250

Mix well, 90℃ water bath for 10 minutes, then 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 -AL (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-AL (U/min/mg prot) =x×Vs÷(Cpr×Vs) ÷T= 0.2×x÷Cpr

Vs: Sample volume in reaction system, 0.25 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 1.0, 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:

[1] Sun M, Ma B, Yuan S, Xin L, Zhao C, Liu H. Mercury thermometer-inspired test strip for concentration cell-based potentiometric detection of salivary 伪 -amylase. Anal Chim Acta. 2022 May 8;1206:339770. doi: 10.1016/j.aca.2022.339770. Epub 2022 Apr 1. PMID: 35473854.

[2] Liu H, Yuan L, Guo W, Wu W. Transcription factor TERF1 promotes seed germination under osmotic conditions by activating gibberellin acid signaling. Plant Sci. 2022 Sep;322:111350. doi: 10.1016/j.plantsci.2022.111350. Epub 2022 Jun 13. PMID: 35709980.

[3] Xiao K, Song L, Li Y, Li C, Zhang S. Dietary intake of microplastics impairs digestive performance, induces hepatic dysfunction, and shortens lifespan in the annual fish Nothobranchius guentheri. Biogerontology. 2023 Apr;24(2):207-223. doi: 10.1007/s10522-022-10007-w. Epub 2023 Jan 2. PMID: 36592268.

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[5] Wu A, Li L, Zhang S, Lin Q, Liu J. Optimization of the hongqu starter preparation process for the manufacturing of red mold rice with high gamma-aminobutyric acid production by solid-state fermentation. Biotechnol Appl Biochem. 2023 Feb;70(1):458-468. doi: 10.1002/bab.2370. Epub 2022 Jun 12. PMID: 35662255.

References:

[1] Hashemi M, Mousavi S M, Razavi S H, et al. Comparison of submerged and solid state fermentation systems effects on the catalytic activity of Bacillus sp. KR-8104 α-amylase at different pH and temperatures[J]. Industrial crops and products, 2013, 43: 661-667.

[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
BC1850/BC1855	Soluble Starch Synthase(SSS) Activity Assay Kit
BC3290/BC3295	Bound Station amylosynthease Activity Assay Kit