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# α-Amlase(α-AL) Activity Assay Kit

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

**Detection instrument:** Spectrophotometer

**Cat No:** AK0532

**Size:** 50T/24S

**Components:**

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

**Reagent II:** Powder×1. Store at 4℃ . Add 20 mL of distilled water when the solution will be used, place in room temperature water, heat with frequent agitation and boil to completely dissolve the powder.

**Standard:** Powder×1,10 mg anhydrous glucose. Add 1 mL of distilled water to form 10 mg/mL glucose standard solution when the solution will be used.

**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 Dilution of standard: 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(mg/mL) | - | - | 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. $A_{\text{control tube}}$=A(C), $A_{\text{blank tube}}$=A(B), $A_{\text{test tube}}$=A(T), $A_{\text{Standard tube}}$=A(S), ΔA (T)=A(T)-A(C), ΔA (S)=A(S)-A(B)

**III. Calculation:**

1 Create standard curve

Using the concentration of standard solution as x axis and ΔA(S) as y axis create standard curve, obtain equation y=kx+b. Put ΔA into the equation and obtain the 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] Yu Z, Yang Z, da Silva J A T, et al. Influence of low temperature on physiology and bioactivity of postharvest Dendrobium officinale stems[J]. Postharvest biology and technology, 2019, 148: 97- 106.

[2] Chen M X, Zhu F Y, Wang F Z, et al. Alternative splicing and translation play important roles in hypoxic germination in rice[J]. Journal of experimental botany, 2019, 70(3): 817-833.

[3] QinYuan, ShangLin, YuanFu, et al. Effects of extraction methods on the physicochemical characteristics and biological activities of polysaccharides from okra (Abelmoschus esculentus). International Journal of Biological Macromolecules. November 2018;(IF3.909)

**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.

**Related Products：**

AK0321/AK0320 ADPG Pyrophosphorylase(AGP) Activity Assay Kit

AK0413/AK0615 Soluble Starch Synthase(SSS) Activity Assay Kit

AK0364/AK0363 Bound Station amylosynthease Activity Assay Kit