Ver.251201

# α- Amylase (α -AL) Activity Assay Kit

BC12027-01 (50T/24S)

**FOR RESEARCH USE ONLY, DO NOT USE IT IN CLINICAL DIAGNOSIS**

# Product Description

Amylase including α-amylase and β-amylase. α-amylase 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°C for 15 minutes. Therefore, only α -AL could catalyze starch hydrolysis when the crude enzyme solution is passivated at 70°C for 15 minutes.

## Kit components

| Reagent | Volume | Storage |
|---|---|---|
| Reagent I | 40mL | RT |
| Reagent II | 10mL x 2 | 2-8°C |
| Reagent III | Powder x 2 | 2-8°C |
| Standard | Powder x 1 | 2-8°C |

## Solution preparation:

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

2. **Reagent II:** 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 2-8°C for four weeks.

3. **Standard:** 10mg 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 2-8°C for two weeks.

# Reagents and Equipment Required but Not Provided

Spectrophotometer, desk centrifuge, water bath, adjustable pipette, mortar/homogenizer/cell ultrasonic crusher, 1 mL glass cuvette, and distilled water.

# Protocol

**1. Sample Extraction**: (The sample size to be tested can be adjusted appropriately, and the specific proportion can be referred to the literature.)

It is suggested that weigh about 0.1g 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 6000x 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.

## 2. Determination operation

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

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

III. Take 250 μL of sample and take a boiling bath for 5 minutes which use as control tube. (Wrap the sealing film to prevent bursting.)

IV. 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 (Boiled sample) | 250 | - | - |
| Distilled water | - | - | - | 250 |
| Standard solution | - | - | 250 | - |
| Incubate at 70°C water bath for 15 minutes, cooling | | | | |
| Reagent II | - | 250 | - | - |
| Incubate at 40°C water bath for 5 minutes | | | | |
| Reagent I | 500 | 500 | 500 | 500 |
| Reagent II | 250 | - | 250 | 250 |

Mix well, boiling water bath for 10 minutes, then measure the absorbance at 540 nm. Record as $A_T$. Ac, As, $A_B$, and calculate $\Delta A_S = A_S - A_B$, $\Delta A_T - A_T - A_C$. Each test tube should be provided with one contrast tube. Standard curve and blank tube only need to be measured once or twice.

## 3. Calculation

### I. Create standard curve

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

### II. Enzyme activity calculation

a) 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.

**$\alpha$-AL activity(U/g mass) = x×Vs ÷ (W×Vs÷Ve) ÷T=2 ×x ÷W**

b) 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.

**$\alpha$-AL activity(U/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 mass, 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.