# β-amylase(β-AL) Activity Assay Kit

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

**Detection instrument:** Spectrophotometer

**Cat No:** BC2040

**Size:** 50T/24S

## Components:

**Reagent I:** Liquid 65 mL×1. Store at room temperature. If yellow crystal is precipitated, heated moderately to dissolve before use.

**Reagent II:** Liquid 18 mL×2. Store at 2-8℃. 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℃ for four weeks.

**Reagent III:** Powder×2. Store at 2-8℃.

**Standard:** Powder×1. Store at 2-8℃. 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 2-8℃ for two weeks.

## Product Description:

Amylase is responsible for hydrolyzing starch, including α-amylase and β-amylase. β-amylase (EC 3.2.1.2) cuts α-1, 4 glycoside bonds from the non-reducing end of starch to produce glucose, maltose, maltose, dextrin and other reducing sugars.

Reducing sugar reduced 3,5-dinitrosalicylic acid to form brown red substance. α-amylase is acid-resistant and β-amylase is heat-resistant. According to the above characteristics, the activity of another amylase can be measured by passivating one of them.

## Required material：

Spectrophotometer, thermostat water bath, centrifuge, transferpettor, 1 mL glass cuvette, 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, place the extract at room temperature and extract 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 constant volume to 10 mL, shake well, that is the original amylase solution.

Take 1 mL of the above-mentioned amylase stock solution, add 4 mL of distilled water, shake well, it is the diluted amylase solution, which is used for the determination of the total activity of (α+β) amylase.

### 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,

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0.025, 0.0125 and 0.00625 mg/mL.

3、Add 250 μL of amylase stock solution and diluted amylase solution into two EP tubes respectively, and use them as the contrast tube of α-amylase contrast tube and the contrast tube of β-amylase respectively after boiling for 5 minutes.

4、Measurement operation table:

| Reagent (μL) | Measured of α-amylase activity | | Measured of total amylase activity | | Measured of standard curve | |
|---|---|---|---|---|---|---|
| | Contrast tube (C) | Test tube (T) | Contrast tube (C) | Test tube (T) | Blank tube (B) | Standard tube (S) |
| Amylase stoste | 250 (boiling) | 250 | - | - | - | - |
| Distilled water | - | - | - | - | 250 | - |
| Standard solution | - | - | - | - | - | 250 |
| Incubate in 70℃ water bath for 15 minutes, cooling. | | | | | | |
| diluted amylase solution | - | - | 250(boiling) | 250 | - | - |
| Reagent II | - | 250 | - | 250 | - | - |
| Incubate in 40℃ thermostat water bath for 5 minutes. | | | | | | |
| Reagent I | 500 | 500 | 500 | 500 | 500 | 500 |
| Reagent II | 250 | - | 250 | - | 250 | 250 |

Mix well, boiling water bath for 10 minutes, measure the absorbance at 540 nm. recorded as $A_1$, $A_2$, $A_3$, $A_4$, $A_5$ and $A_6$ respectively from left to right. $\Delta A_\alpha = A(2) - A(1)$, $\Delta A_{Total} = A(4) - A(3)$, $\Delta A_{Standard} = A(6) - A(5)$. 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

Using the concentration of standard solution as x axis and $\Delta A_S$ as y axis create standard curve, obtain equation $y = kx + b$. Put $\Delta A_\alpha$ into the equation and obtain the $x_1$(mg/mL), Put $\Delta A_{Total}$ into the equation and obtain the $x_2$(mg/mL).

2、Calculation of α-amylase activity

(1) Calculation according to sample quality

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the generation of 1 mg of reducing sugar in the reaction system per minute every g sample.

α-amylase activity (U/g fresh weight) $= x_1 \times V_S \div (W \times V_S \div V_{ST}) \div T = 2 \times x_1 \div W$

(2) Calculation according to protein content

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the generation of 1 mg of reducing sugar in the reaction system per minute every mg protein.

α-amylase activity (U/mg prot) $= x_1 \times V_S \div (V_S \times Cpr) \div T = 0.2 \times x_1 \div Cpr$

3、Calculation of total amylase activity

(1) Calculation according to sample quality

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the

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generation of 1 mg of reducing sugar in the reaction system per minute every g sample.

Total amylase (U/g fresh weight)=$5 \times x_2 \times V_S \div (W \times V_S \div V_{ST}) \div T = 10 \times x_2 \div W$

(2) Calculation according to protein content

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the generation of 1 mg of reducing sugar in the reaction system per minute every mg protein.

Total amylase (U/mg prot) = $5 \times x_2 \times V_S \div (V_S \times Cpr) \div T = x_2 \div Cpr$

4、Calculation of β-amylase activity

(1) Calculation according to sample quality

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the generation of 1 mg of reducing sugar in the reaction system per minute every g sample.

β-amylase activity (U/g fresh weight) = The activity of total amylase - α-amylase activity
$$= (10 \times x_2 \div W) - (2 \times x_1 \div W) = (10 \times x_2 - 2 \times x_1) \div W$$

(2) Calculation according to protein content

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the generation of 1 mg of reducing sugar in the reaction system per minute every mg protein.

β-amylase activity (U/mg prot) = The activity of total amylase - α-amylase activity
$$= (x_2 \div Cpr) - (0.2 \times x_1 \div Cpr)$$

5: Dilution ratio of total amylase, (4 mL+1 mL)/1 mL=5;

$V_S$: The volume of sample added to reaction system, 0.25 mL;

$V_{ST}$: Total volume of extract, 10 mL;

Cpr: Sample protein concentration, mg/mL;

T: Reaction time, 5 minutes;

W: Sample weight, g.

**Note:**

When the measured absorbance value is greater than 1, the sample can be appropriately diluted for determination. If the absorbance value is too small, diluted amylase solution or amylase stock solution can be concentrated.

**References：**

[1] Dziedzoave N T, Graffham A J, Westby A, et al. Influence of variety and growth environment on β-amylase activity of flour from sweet potato (Ipomea batatas)[J]. Food control, 2010, 21(2): 162-165.

**Related Products：**

BC0700/BC0705    Glutamate Synthase(GOGAT) Activity Assay Kit
BC0610/BC0615    α-amylase Activity Assay Kit
BC0430/BC0435    ADPG Pyrophosphorylase(AGP) Activity Assay Kit