



Ver. 25061

## **Amylose Content Assay Kit**

BC4260-01 (50 Tests/48 Samples)

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

## Product Description

Amylose is a polysaccharide chain linked by d-glucosyl -(1,4) glycosidic bonds, which affects the edible quality and appearance quality of food, and is closely related to food safety.

Amylose reacts with iodine to form a blue complex, which results in colour formation proportional to the amount of amylose. Soluble sugar and starch in the sample are separated using ethanol before measuring the amylose content by reacting with iodine.

## Kit components

Reagent	Volume	Storage
Reagent I	50mL	4°C
Reagent II (Ether)	50mL Not provided	4°C
Reagent III	20mL	4°C
Reagent IV		
Reagent III and distilled water are mixed by the ratio of 9 mL: 91 mL to make Reagent IV. Prepare only what is required as per the number of samples. Storage at 4°C and use within 6 months of reconstitution.		
Reagent V	5mL	4°C
Reagent VI	5mL	4°C
Standard (10mg amylose)	Powder	4°C
Add 0.1 mL of absolute ethanol and 0.9 mL of Reagent III before use. Covering the lid, boiling until it fully dissolved to produce a 10 mg/mL amylose standard solution. Take 0.1 mL of 10 mg/mL amylose standard solution and add 0.9 mL of distilled water to prepare 1 mg/mL Working Standard. The Amylose standard solution (10 mg/mL) could be stored at 4°C for six months. Working standard (1 mg/mL) should be prepared freshly before each experiment.		

## Reagents and Equipment Required but Not Provided

Spectrophotometer, ice, desk centrifuge, adjustable pipette, 1 mL glass cuvette, mortar/homogenizer, ether, absolute ethanol, ice and distilled water.

## Protocol

### I. Sample Preparation:

Weigh 0.01 g of dried sample and grind it in a mortar, add 0.1 mL of 95% Ethanol and 0.9 mL of Reagent I and transfer to 1.5mL microcentrifuge tube after complete homogenization. Incubate at 80°C for 30 minutes. Cool to room temperature in an ice bath. Centrifuge at 3000×g for 5 minutes at 25°C. Discard the supernatant and add 1mL Reagent II (ether). Centrifuge at 3000×g for 5 minutes at 25°C. Discard the supernatant. Dissolve the sediment in 5mL Reagent IV and incubate at 90°C for 10 minutes. Cool to room temperature in an ice bath. Centrifuge at 3000×g for 5 minutes at 25°C. Collect the supernatant for the assay.

### II. Determination procedure:

- Preheat the spectrophotometer for 30 minutes, adjust wavelength to 600nm, set zero with distilled water
- Dilute Working Standard (1mg/mL) to 0.4 mg/mL with Reagent IV.
- Add reagents in a 1mL glass cuvette as mentioned in the table:

Reagent(μL)	Test (T)	Standard (S)	Blank (S)
Sample	100	-	-
Working Standard (1 mg/mL)	-	100	-
Distilled Water	-	-	100
Reagent V	20	20	20
Reagent VI	20	20	20
Distilled Water	860	860	860

- Mix well, take the supernatant to measure absorption at 600nm.
- Under the 600 nm, record as  $A_T$ ,  $A_S$  and  $A_B$  respectively.
- Calculate,  
 $\Delta A_T = (A_T - A_B)$   
 $\Delta A_S = (A_S - A_B)$

## Calculation

$$\text{Amylose content (mg/g weight)} = \Delta A_T \div (\Delta A_S \div C_s) \times V_e \div W$$

$$= 5 \times \Delta A_T \div \Delta A_S \div W$$

$C_s$  : Standard concentration, 1mg/mL

$V_e$  : Reagent IV volume, 5 mL.

$W$  : Sample weight, g.

## Note:

- If  $A_T < 0.03$  or  $A_T$  is close to blank tube, the volume of Reagent IV can be decreased during extraction. If  $A_T > 1$ , the sample can be appropriately diluted with Reagent IV. Appropriate modifications have to be made in the calculation.
- It is recommended to measure the absorbance within 20 minutes to prevent fading of the colour.