(FOR RESEARCH USE ONLY. DO NOT USE IT IN CLINICAL DIAGNOSIS!)

Amylose Content Assay Kit

Catalog No.: BC00172

Size: 50T

Please read the instructions carefully before use. If you have any questions or need further help during experiment, please don't hesitate to contact us through the following methods:

✉ Email (Sale) order@enkilife.com

✉ Email (Techsupport) techsupport@enkilife.com

☎ Tel: 0086-27-87002838

Website: www.enkilife.com

Shelf life: Please refer to the label on the outer package.

Techsupport: In order to provide you with better service, please inform us the lot number on the label of the outer package.

Basic Information

Product Name	Amylose Content Assay Kit
Detection Method	Colorimetric
Assay Type	Quantitative
Detection Instrument	Spectrophotometer (620 nm)

Product Introduction

Amylose is a polysaccharide chain linked by D-glucosyl units via α-(1,4) glycosidic bonds. Its quantitative determination is of great significance for evaluating the nutritional value of food and investigating carbohydrate metabolism in plants.

Principle

80% ethanol can separate soluble sugars from starch in samples. The complex formed by amylose and iodine exhibits an absorption peak at 620 nm.

Components

No.	Components	Size	Notes
Reagent 1	Liquid	50 mL	Store at 4°C
Reagent 2	Ether or petroleum ether	analytical grade	Self-provided
Reagent 3	Liquid	60 mL	Store at 4°C
Reagent 4	Liquid	5 mL	Store at 4°C
Reagent 5	Liquid	1 mL	Store at 4°C away from light
Reagent 6	Amylopectin standard	10 mg	Store at 4°C

Storage

The kit has a validity period of 3 months.

Preparation

Visible spectrophotometer, water bath, adjustable pipette, 1 mL cuvette, ether (or petroleum ether) and distilled water.

- **Sample handling**

1. Dry Samples

Grind the dry sample thoroughly into fine powder in a mortar (or grind under liquid nitrogen). Weigh 0.01-0.02 g (approximately 0.01 g is recommended) into an EP tube, add 1 mL of Reagent 1, and vortex fully for about 30 seconds. Perform extraction in an 80 °C water bath for 30 min, then centrifuge at 4000 rpm for 5 min at room temperature. Discard the supernatant and retain the precipitate. Add 1 mL of Reagent 2, shake and mix well for 5 min, followed by centrifugation at 4000 rpm for 5 min at room temperature. Discard the supernatant and keep the precipitate. If residual Reagent 2 cannot be completely aspirated accurately, place the EP tube in a 37 °C oven for 5-10 min and take it out afterward. Add 1 mL of Reagent 3, mix thoroughly, dissolve in a 90 °C water bath for 10 min, cool down, and set aside for measurement.

2. Fresh Samples

Weigh approximately 0.1 g of fresh sample, add 1 mL of Reagent 1, and homogenize by grinding in a sealed container. Transfer the homogenate to an 80 °C water bath for 30 min extraction, then centrifuge at 4000 rpm for 5 min at room temperature. Discard the supernatant and retain the precipitate. Add 1 mL of Reagent 2, shake and mix well for 5 min, then centrifuge at 4000 rpm for 5 min at room temperature. Discard the supernatant and keep the precipitate. If residual Reagent 2 cannot be completely aspirated accurately, place the EP tube in a 37 °C oven for 5-10 min and take it out afterward. Add 1 mL of Reagent 3, mix thoroughly, dissolve in a 90 °C water bath for 10 min, cool down, and set aside for measurement.

Operation process

	Blank tube	Standard tube	Assay tube
Reagent 3 (μL)	100		
Standard Working Solution (μL)		100	
Samples (μL)			100

Reagent 4 (μL)	70	70	70
Distilled water (μL)	620	620	620
Reagent 5 (μL)	10	10	10
Distilled water (μL)	200	200	200
Mix thoroughly, measure the absorbance of each tube at a wavelength of 620 nm using a 1 mL cuvette, with distilled water used for zero calibration.			

Calculation

1. Preparation of standard curve: Add 1 mL of Reagent 3 to one vial of standard powder and vortex thoroughly until completely dissolved to obtain a 10 mg/mL standard working solution. Dilute the 10 mg/mL standard solution with Reagent 3 to concentrations of 0.05, 0.1, 0.2, 0.4, 0.6, 0.8 and 1.0 mg/mL respectively for preparing the standard curve.
2. Calculated based on sample mass

$$\text{Amylopectin Content (mg/g)} = \frac{A_{Assay} - A_{Blank} - 0.0261}{2.4448} \times V \div W$$

3. Calculated based on sample protein concentration

$$\text{Amylopectin Content (mg/g)} = \frac{A_{Assay} - A_{Blank} - 0.0261}{2.4448} \div Cpr$$

Annotation:

V: Total volume of extract obtained from sample pretreatment, 1 mL

W: Sample mass, g

Cpr: Protein concentration of the test sample

Precautions

1. It is recommended to use disposable laboratory test tubes for operation.
2. This product is intended for scientific research use only by professionals and must not be used for clinical diagnosis or treatment, in food or drugs, or stored in ordinary residences.
3. Prior to adding Reagent 3 during sample pretreatment, ensure ether (or petroleum ether) is fully

evaporated. Vortex thoroughly to disperse the precipitate for complete dissolution after incubation in a 90 °C water bath.

4. Ether (or petroleum ether) is difficult to aspirate; glass pipettes are recommended. Minor volumetric errors will not affect experimental results.
5. The standard is pure amylose, which can be dissolved at room temperature after adding Reagent 3, and the standard curve only needs to be prepared once.
6. After sample pretreatment, select 1 to 2 samples with greatly different concentrations for a preliminary test to determine the optimal sample concentration. It is generally recommended to test samples with an OD value ≤ 1.
7. If the microplate reader is equipped with the corresponding wavelength, transfer 200-300 μL of the final chromogenic solution to a 96-well plate to measure absorbance. In this case, the standard curve must be prepared and measured by the same method.