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

Amylopectin Content Assay Kit

Catalog No.: BC00173

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	Amylopectin Content Assay Kit
Detection Method	Colorimetric
Assay Type	Quantitative
Detection Instrument	Spectrophotometer (550 nm and 743 nm)

Product Introduction

Amylopectin is a highly branched polysaccharide. The proportion and content of amylose and amylopectin in starch exert a direct impact on the processing performance, physicochemical properties, gelatinization temperature and other characteristics of starch products. Therefore, research on starches with varying amylose/amylopectin ratios is of great significance.

Principle

80% ethanol can be used to separate soluble sugars from starch in samples, and the dual-wavelength colorimetric method is adopted to determine the amylopectin content.

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
Reagent 5 (μL)	10	10	10
Distilled water (μL)	820	820	820
Mix well, incubate in the dark at room temperature for 10 minutes. Use a 1 mL cuvette and set zero with distilled water, then measure the absorbance of each tube at 550 nm and 743 nm respectively.			

Calculation

1. Preparation of standard curve: Add 1 mL of Reagent 3 to one vial of standard powder, vortex thoroughly until fully dissolved to prepare the 10 mg/mL standard working solution. Further dilute the 10 mg/mL standard solution with Reagent 3 to concentrations of 0.2, 0.4, 0.6, 0.8, 1, 1.5, 2 and 2.5 mg/mL for plotting the standard curve.
2. Calculated based on sample mass (Recommended)

$$\text{Amylopectin Content (mg/g)} = \frac{A_{Assay} - A_{Blank} + 0.0067}{0.18} \times V \div W$$

3. Calculated based on sample protein concentration

$$\text{Amylopectin Content (mg/g)} = \frac{A_{Assay} - A_{Blank} + 0.0067}{0.18} \div Cpr$$

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 amylopectin, which dissolves at room temperature after adding Reagent 3. Gentle heating can speed up dissolution if needed. The standard curve only needs to be prepared once.
6. After sample pretreatment, conduct a preliminary test with 1-2 samples with significantly different concentrations to determine the optimal sample concentration. It is generally recommended that the OD value of samples for formal measurement be around 0.2.
7. Amylopectin produces a reddish-brown color after color reaction. Color interference from amylose may mask the characteristic hue during sample testing, yet this does not interfere with absorbance measurement.
8. If the microplate reader supports the required wavelengths, transfer 200-300 μL of the final chromogenic solution to a 96-well plate and read absorbance at 550 nm and 743 nm. In this case, a standard curve must be prepared and measured using the identical procedure.