Anthocyanin Content Assay Kit - Spectrophotometric Method

Product Code: 112708

Product Introduction

Anthocyanins are natural pigments that are readily soluble in polar solvents and belong to the flavonoid class of compounds. They are widely present in the roots, stems, leaves, flowers, and fruits of plants, producing colors that range from red to purple.

This kit uses the pH differential method to determine anthocyanin content. At pH 1.0, anthocyanins show a maximum absorption peak at 530 nm. At pH 4.5, anthocyanins convert to the colorless chalcone form and show no absorption peak at 530 nm. Based on this property, absorbance is measured at 530 nm and 700 nm under different pH conditions. This method reduces the effects of solution pH and solvent differences and helps eliminate interference from non-anthocyanin substances.

Reference test data: sample blueberry. A1 OD values: 0.618, 0.586; A2 OD values: 0.002, 0.003; A3 OD values: 0.044, 0.046; A4 OD values: 0.002, 0.001. Actual readings may vary depending on the instrument used. These values are for reference only.

Package Contents

Pack Size	Code	Item	Specification
50T	112708.1	Reagent I	50 mL
50T	112708.2	Reagent II	50 mL
50T	112708.3	Extraction Solution	50 mL
50T	112708.m	Manual	1 copy

Quality Standards and Safety Instructions

Raw Material and Packaging Name	Quality Standard	Main Toxicity
Reagent I	--	--
Reagent II	--	--
Extraction Solution	--	--

Transportation and Storage

Transport: Transport with ice packs.

Storage: Store at 2-8℃.

Shelf life: 180 days.

Instructions for Use

1. Anthocyanin Extraction

1. Use a sample mass (g) to extraction solution volume (mL) ratio of 1:5 to 1:10.
2. It is recommended to weigh about 0.1 g of sample and add 1 mL of extraction solution.
3. Homogenize thoroughly and transfer to an EP tube.
4. Make up the volume to 1 mL with extraction solution, seal tightly, and ultrasonically extract for 2 h.
5. Centrifuge at 8000g at room temperature for 10 min.

6. Collect the supernatant for testing.

2. Assay Procedure

1. Preheat the spectrophotometer for at least 30 min. Preheat Reagent I and Reagent II at 25℃ (room temperature) for at least 10 min.
2. Mix 100 μL of supernatant with 900 μL of Reagent I (10-fold dilution). Incubate in a 40℃ water bath for 20 min, then measure absorbance at 530 nm and 700 nm. Record these as A1 and A2.
3. Mix 100 μL of supernatant with 900 μL of Reagent II (10-fold dilution). Incubate in a 40℃ water bath for 20 min, then measure absorbance at 530 nm and 700 nm. Record these as A3 and A4.
4. Calculate ΔA = (A1 - A2) - (A3 - A4).

If A1 is greater than 1, increase the dilution factor while keeping the total volume at 1 mL unchanged. For example, use 50 μL supernatant and 950 μL Reagent I (20-fold dilution). If A1 is less than 0.1, reduce the dilution factor while keeping the total volume unchanged. For example, use 200 μL supernatant and 800 μL Reagent I (5-fold dilution). Keep A1 within the 0.1-1 range to improve detection sensitivity. Adjust the volume ratio of supernatant and Reagent II in the same way. Use the actual dilution factor in the calculation formula below.

3. Content Calculation

Anthocyanin content (μg/g fresh weight) = $[\Delta A \times V \div (\varepsilon \times d) \times M \times F \times 10^6] \div W$

$= 16.7 \times \Delta A \times F \div W$

Symbol	Definition
V	Extraction solution volume, 1×10^{-3}L
ε	Molar extinction coefficient of anthocyanin, 2.69×10^4L/mol/cm
d	Cuvette path length, 1 cm
M	Relative molecular mass of anthocyanin, 449.2 g/mol
F	Dilution factor
10^6	1 g = 10^6μg
W	Sample dry weight, g

Precautions

1. Before the formal assay, select 2-3 samples with large expected differences for a preliminary test.
2. Required instruments and supplies not provided: visible spectrophotometer, water bath, adjustable pipette, 1 mL glass cuvette, ultrasonic cleaner, mortar, and distilled water.
3. The linear range of ΔA is 0.005-1.
4. This product is intended for scientific research use by professionals only. It must not be used for clinical diagnosis or treatment, food, or drugs, and must not be stored in ordinary residences.
5. For safety and health, wear a lab coat and disposable gloves during operation.