Anthocyanin Assay Kit (Spectrophotometry)

Description

Anthocyanins are a class of naturally occurring pigments that are easily soluble in polar solvents and belong to the flavonoid family. They are widely distributed in the roots, stems, leaves, flowers, and fruits of plants, giving them colors ranging from red to purple. Anthocyanins are the primary pigments responsible for the coloration of plants.

Detection Principle

Determination of anthocyanin content by the pH differential method: At pH 1.0, anthocyanins exhibit a maximum absorption peak at 530 nm, whereas at pH 4.5, anthocyanins convert to the colorless chalcone form and show no absorption at 530 nm. This property is utilized to measure the absorbance values at 530 nm and 700 nm under different pH conditions. The pH differential method reduces the influence of solution pH and solvent differences and eliminates interference from non-anthocyanin substances in the measurement.

Product Information

E.g. 50T/48S pack:

Components	Packing Size	Storage
CB0027S-ES	50mL ×1	2-8℃
CB0027S-A	50mL ×1	2-8℃
CB0027S-B	50mL ×1	2-8℃

Note: Select 2-3 samples with expected large differences for a preliminary test.

Instructions

I. Equipment For Use

Visible spectrophotometer;
Water bath, adjustable micropipette;
1 mL glass cuvette;
Mortar and pestle;
Distilled water.

II. Anthocyanin Extraction

Use a ratio of dried sample weight (g) to CB0027S-ES volume (mL) of 1:5-10 (* it is recommended to weigh approximately 0.1 g of dried sample and add 1 mL of CB0027S-ES). Homogenize thoroughly and transfer to an EP tube. Adjust the volume of CB0027S-ES to 1 mL, cap tightly, and extract at 4 ℃ for 24 h. Centrifuge at 8000 g, 25 ℃ for 10 min, and collect the supernatant for measurement.

III. Measurement Procedure:

1. Preheat the spectrophotometer for at least 30 min; preheat CB0027S-A and CB0027S-B for at least 10 min.
2. Take 100 µL of supernatant and add 900 µL of CB0027S-A (10×dilution), incubate in a 40 ℃ water bath for 20 min, then measure absorbance at 530 nm and 700 nm. Record as A1 and A2, respectively.
3. Take 100 µL of supernatant and add 900 µL of CB0027S-B (10×dilution), incubate in a 40 ℃ water bath for 20 min, then measure absorbance at 530 nm and 700 nm. Record as A3 and A4, respectively.
4. Calculate ΔA = (A1 -A2)-(A3-A4).

Note:

- If A1 > 1, you can increase the dilution while keeping the total volume at 1 mL (e.g., 50 µL supernatant + 950 µL CB0027S-A, 20× dilution).
- If A1 < 0.1, you can decrease the dilution while keeping the total volume at 1 mL (e.g., 200 µL supernatant + 800 µL CB0027S-A, 5× dilution) to keep A1 within 0.1 – 1 and improve detection sensitivity.
- Adjust the supernatant and CB0027S-B volume proportion similarly. Use the actual dilution factor in the calculation.

Calculation of Anthocyanin Content

Anthocyanin content (µg/g dry weight)=[ΔA×V÷(ε×d)×M×F×106]÷W=16.7×ΔA× F÷W

V: volume of CB0027S-A, 1 × 10^{-3} L

ε: molar extinction coefficient of anthocyanin, 2.69 × 10^{4} L/mol/cm

d: optical path length of cuvette, 1 cm

M: relative molecular weight of anthocyanin, 449.2 g/mol

F: dilution factor

10^{6}: conversion factor, 1 g = 10^{6} µg

W: dry weight of sample, g

Precautions

1. The product is for R&D use only, not for diagnostic procedures, food, drug, household or other uses.
2. Please wear a lab coat and disposable gloves.