

**Anthocyanin Content Assay Kit - Micro Method (112709)****Product Introduction**

Anthocyanins are natural pigments that are readily soluble in polar solvents and belong to the flavonoid class of compounds. They are widely found in plant roots, stems, leaves, flowers, and fruits, where they produce colors ranging from red to purple and serve as major plant pigments.

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 minimizes interference from non-anthocyanin substances.

Reference example: when the sample was blueberry, A1 OD values were 0.408 and 0.388; A2 OD values were 0.047 and 0.046; A3 OD values were 0.093 and 0.090; A4 OD values were 0.048 and 0.047. Actual readings may vary depending on the instrument used.

**Package Contents**

Pack Size	Code	Item	Volume
100T	112709.1	Reagent I	20 mL
100T	112709.2	Reagent II	20 mL
100T	112709.3	Extraction Solution	100 mL
100T	112709.m	Manual	1 copy

**Quality Standards and Safety Information**

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

**Transportation and Storage**

Condition	Requirement
Transportation	Transport with ice packs.
Storage	Store at 2-8°C.
Shelf Life	180 days

**Instructions for Use****1. Anthocyanin Extraction**

Use an extraction solution volume to sample mass ratio of 1:5-10 (mL:g). It is recommended to weigh about 0.1 g of sample and add 1 mL of extraction solution.

1. Homogenize the sample thoroughly and transfer it to an EP tube.
2. Make up to 1 mL with extraction solution.
3. Seal tightly and ultrasonically extract for 2 h.

4. Centrifuge at 8000g at room temperature for 10 min.
5. Collect the supernatant for testing.

## 2. Assay Procedure

1. Preheat the spectrophotometer or microplate reader for more than 30 min. Preheat Reagent I and Reagent II at 25°C (room temperature) for more than 10 min.
2. Mix 20 µL of supernatant with 180 µL of Reagent I (10-fold dilution). Incubate in a 40°C water bath for 20 min, then measure the absorbance at 530 nm and 700 nm. Record the values as A1 and A2.
3. Mix 20 µL of supernatant with 180 µL of Reagent II (10-fold dilution). Incubate in a 40°C water bath for 20 min, then measure the absorbance at 530 nm and 700 nm. Record the values as A3 and A4.
4. Calculate  $\Delta A = (A1 - A2) - (A3 - A4)$ .

If A1 is greater than 1, increase the dilution factor while keeping the total volume at 200 µL unchanged. For example, use 10 µL supernatant and 190 µL Reagent I for a 20-fold dilution. If A1 is less than 0.1, reduce the dilution factor while keeping the total volume unchanged. For example, use 100 µL supernatant and 100 µL Reagent I for a 2-fold dilution. Adjust the volume ratio of the supernatant and Reagent II in the same way. Use the actual dilution factor in the calculation formula. Keeping A1 within the 0.1-1 range can improve detection sensitivity.

## 3. Content Calculation

$$\text{Anthocyanin content } (\mu\text{g/g fresh weight}) = [\Delta A \times V \div (\epsilon \times d) \times M \times F \times 10^6] \div W$$

$$= 33.4 \times \Delta A \times F \div W$$

Symbol	Definition
V	Extraction solution volume, $1 \times 10^{-3}$ L
$\epsilon$	Molar extinction coefficient of anthocyanin, $2.69 \times 10^4$ L/mol/cm
d	Cuvette path length, 0.5 cm
M	Relative molecular mass of anthocyanin, 449.2 g/mol
F	Dilution factor
$10^6$	$1 \text{ g} = 10^6 \mu\text{g}$
W	Sample dry weight, g

## Notes

1. Before the formal assay, select 2-3 samples with large expected differences for pre-testing.
2. Required instruments and supplies: visible spectrophotometer or microplate reader, water bath, adjustable pipette, 1 mL glass cuvette or 96-well plate, ultrasonic cleaner, mortar, and distilled water.
3. The linear range of  $\Delta A$  is 0.005-1.
4. This product is for scientific research use by professionals only. It must not be used for clinical diagnosis or treatment, must not be used in 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.