**Anthocyanin Content Assay Kit - Microplate Method**

Product code: 112710

## 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 the roots, stems, leaves, flowers, and fruits of plants, where they produce colors ranging from red to purple and are the main pigments responsible for plant coloration.

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, they are converted to a 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.

The pH differential method reduces the effects of solution pH variation and solvent differences, helping eliminate interference from non-anthocyanin substances.

Reference assay results for a blueberry sample: A1 OD values 0.408 and 0.388; A2 OD values 0.047 and 0.046; A3 OD values 0.093 and 0.090; A4 OD values 0.048 and 0.047. Actual readings may vary depending on the instrument and test conditions.

## Package Contents

| Pack Size | Code | Item | Volume / Quantity |
|---|---|---|---|
| 100T | 112710.1 | Reagent I | 20 mL |
| 100T | 112710.2 | Reagent II | 20 mL |
| 100T | 112710.3 | Extraction Solution | 100 mL |
| 100T | 112710.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

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

## Instructions for Use

### 1. Anthocyanin Extraction

1. Use an extraction solution volume to sample mass ratio of 1:5 to 1:10 (mL:g).
2. It is recommended to weigh about 0.1 g of sample and add 1 mL of extraction solution.
3. Fully homogenize and transfer to an EP tube.

4. Adjust the extraction solution volume to 1 mL, cap tightly, and perform ultrasonic extraction 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 microplate reader for at least 30 min. Preheat Reagent I and Reagent II at 25°C (room temperature) for at least 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 absorbance at 530 nm and 700 nm. Record these 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 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 200 μL. 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. 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.

### 3. Content Calculation

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

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

| | |
|---|---|
| V | Extraction solution volume, $1 \times 10^{-3}$ L |
| ε | 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 g = $10^6$ μg |
| W | Sample dry weight, g |

## Precautions

- Before formal testing, select 2-3 samples with large expected differences for a preliminary test.
- Required instruments and supplies not provided: microplate reader, water bath, adjustable pipette, 96-well plate, ultrasonic cleaner, mortar, and distilled water.
- The linear range of ΔA is 0.005-1.
- This product is for scientific research use by professionals only. It must not be used for clinical diagnosis or treatment, and must not be used for food or drugs or stored in ordinary residences.
- For safety and health, wear a lab coat and disposable gloves during operation.