**Plant Chlorophyll Content Assay Kit - Microplate Method**

Product code: 112380

## Product Introduction

Plant chlorophyll is widely present in green plant tissues, and its content is closely related to photosynthesis and nutritional status. Chlorophyll content is an important indicator of plant growth status.

Chlorophyll a and chlorophyll b have maximum absorption at 663 nm and 645 nm, respectively. Chlorophyll a, chlorophyll b, and total chlorophyll content can be calculated using empirical formulas.

## Example Test Data

The following example data are for reference only. Actual readings may vary depending on the testing instrument and testing conditions.

| Sample | Material | OD663 nm | OD645 nm |
|---|---|---|---|
| Sample 1 | Leaf beet | 0.895 / 0.904 | 0.414 / 0.422 |
| Sample 2 | Clover | 0.454 / 0.463 | 0.212 / 0.220 |

## Product Packing List

| Size | Code | Item | Quantity |
|---|---|---|---|
| 100T | 112380.1 | Reagent I | 6 g |
| 100T | 112380.m | Instruction Manual | 1 copy |

## Quality Standards and Safety Information

| Raw Material or Packaging Name | Quality Standard | Main Toxicity |
|---|---|---|
| Reagent I | -- | -- |

## Transportation and Storage

| Condition | Requirement |
|---|---|
| Transportation | Shipped with ice packs. |
| Storage | Store at 2-8℃. Shelf life: 180 days. |

## Instructions for Use

### 1. Preparation of Extraction Solution

Mix 240 mL distilled water with 960 mL acetone thoroughly and set aside.

### 2. Operating Steps

1. Weigh fresh plant leaves or other green tissues. Remove the midrib, weigh approximately 0.1 g, cut into pieces, and rinse clean

with distilled water.
2. Add 1 mL distilled water and a small amount of Reagent I, approximately 10 mg. Grind thoroughly in the dark or under low-light conditions, then transfer to a 10 mL glass test tube.
3. Rinse the mortar with extraction solution. Transfer all rinsing liquid into the glass test tube, then bring the volume to 10 mL with extraction solution.
4. Place the glass test tube in the dark or wrap it with aluminum foil and extract for 3 h.
5. Check whether the tissue residue at the bottom of the test tube has completely turned white. If it has not completely turned white, continue extraction until it does.
6. Transfer 200 μL of the extract into a 96-well plate. Zero with extraction solution and measure absorbance at 663 nm and 645 nm. Record the values as A663 and A645, respectively.

### 3. Calculation of Chlorophyll Content

Chlorophyll a content (mg/g, fresh weight) = (12.7 × A663 - 2.69 × A645) × Vextraction × N ÷ W ÷ 1000 = 0.01 × (12.7 × A663 - 2.69 × A645) × N ÷ W

Chlorophyll b content (mg/g, fresh weight) = (22.9 × A645 - 4.68 × A663) × Vextraction × N ÷ W ÷ 1000 = 0.01 × (22.9 × A645 - 4.68 × A663) × N ÷ W

Total chlorophyll content (mg/g, fresh weight) = (20.21 × A645 + 8.02 × A663) × Vextract × N ÷ W ÷ 1000 = 0.01 × (20.21 × A645 + 8.02 × A663) × N ÷ W

| Symbol | Meaning |
|---|---|
| Vextract | Volume of extract, 10 mL |
| N | Dilution factor |
| W | Sample mass, g |

## Precautions

1. Before formal measurement, select 2-3 samples with large expected differences for preliminary testing. This 100T kit can measure 96 samples.
2. Required instruments and supplies not provided: microplate reader, 96-well plate, adjustable pipette, balance, mortar or homogenizer, aluminum foil, 10 mL test tubes, acetone, and distilled water.
3. Chlorophyll is sensitive to light. Grinding, extraction, and related operations must be performed away from light or under low light.
4. Extraction must continue until the tissue residue turns completely white. Otherwise, extraction will be insufficient.
5. When rinsing the mortar with extraction solution, rinse until all green material is transferred to the glass test tube.
6. If the absorbance value exceeds 1 during measurement, dilute the sample appropriately. If the absorbance value is less than 0.05, the amount of extraction solution may be reduced appropriately to increase the sample concentration. Be sure to modify the Vextract value in the formula accordingly.
7. Acetone is corrosive. If a polystyrene 96-well plate is used for measurement, complete the measurement as soon as possible within 5 min.