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# Plant Chlorophyll Content Assay Kit

Note: Take two or three different samples for prediction before test.

**Operation Equipment:** Spectrophotometer

Catalog Number: AK0302

**Size:** 50T/48S

## **Components:**

Extract: Liquid×1, Required but not provided. Mix anhydrous ethanol: acetone (V: V) = 1:2 for use.

Provide a 125ml empty bottle.

Reagent I: Powder ×1. Storage at 4°C.

### **Product Description**

Chlorophyll is widely found in green plant tissues. It is the organelle of photosynthesis. Its content is closely related to photosynthesis and nutrition. It is an important indicator of plant growth.

The maximum absorption of chlorophyll a and b is at 645 nm and 663 nm. According to the empirical formula, the contents of chlorophyll a, chlorophyll b and total chlorophyll can be calculated.

## Reagents and Equipment Required but Not Provided.

Spectrophotometer, 1 mL glass cuvette, transferpettor, balance, mortar/homogenizer, silver paper, distilled water, anhydrous ethanol and acetone.

#### **Procedure**

## I. Sample preparation:

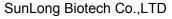
- **1.** Take fresh plant leaves or other green tissues. Wash with distilled water. Absorb surface moisture. Remove the Midrib. Weigh about 0.1 g, cut and put into mortar or homogenizer.
- **2.** Add 1 mL distilled water and a small amount of Reagent I (about 10 mg). Grind well in dark or low light. Transfer to 10 mL test tube.
- 3. Rinse the mortar with the Extract. Transfer all flushing solution into 10 mL EP tube. Use the Extract to fix the volume to 10 mL. Soak in dark or covered with tinfoil for 3 hours. When the color of the bottom tissue residue is close to white, the extraction is complete. If the tissue residue is not completely white. Continue to extract until the color of tissue residue is close to white.

## **II. Determination Procedure**

- 1. Preheat the spectrophotometer for more than 30 minutes, adjust the wavelength to 645 nm and 663 nm, set the counter to zero with Extract.
- 2. Take 1 mL of the upper extract and put it into a 1 mL glass cuvette. Measure the absorbance value at 663 nm and 645 nm, recorded as  $A_{663}$  and  $A_{645}$ , respectively.

# III. Calculation of chlorophyll:

Chlorophyll a content (mg/g fresh weight)= $(12.7 \times A_{663} - 2.69 \times A_{645}) \times V_E \times F \div W \div 1000$ 





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 $=0.01\times(12.7\times A_{663}-2.69\times A_{645})\times F\div W$ 

Chlorophyll b content (mg/g fresh weight)= $(22.9 \times A_{645} - 4.68 \times A_{663}) \times V_E \times F \div W \div 1000$ 

 $=0.01\times(22.9\times A_{645} - 4.68\times A_{663})\times F \div W$ 

Total chlorophyll content (mg/g fresh weight)= $(20.21 \times A_{645} + 8.02 \times A_{663}) \times V_E \times F \div W \div 1000$ 

 $=0.01\times(20.21\times A_{645}+8.02\times A_{663})\times F\div W$ 

V<sub>E</sub>: Extract volume, 10 mL;

W: Sample weight, g;

F: Dilution ratio;

#### Note:

- 1. Chlorophyll is sensitive to light. Grinding and extraction shall be carried out in dark or weak light as far as possible.
- 2. It must be extracted until the tissue residue turns white completely, otherwise the extraction is not sufficient.
- 3. Wash the mortar with the extract until all the green substances are transferred to the EP tube.
- 4. When the absorbance value is more than 1, it can be diluted properly; when the absorbance value is less than 0.05, the amount of  $V_E$  can be reduced properly. Pay attention to change the value of V extraction in the calculation formula.

### **Experimental example:**

Take 0. 1g of chrysanthemum leaf, add 1 mL of distilled water and a small amount of Reagent I (about 10 mg), grind it fully in dark or weak light, and transfer it into 10 mL test tube. Then, according to the operation steps, the content of chlorophyll a  $(mg/g) = 0.01 \times (12.7 \times A663 - 2.69 \times A645) \times F \div W = 0.01 \times (12.7 \times 0.882 - 2.69 \times 0.362) \div W = 1.023 \text{ mg/g}$ ; the content of chlorophyll b  $(mg/g) = 0.01 \times (22.9 \times A645 - 4.68 \times A663) \times F \div W = 0.01 \times (22.9 \times 0.362 - 4.68 \times 0.882) \div W = 0.416 \text{ mg/g}$ ; total chlorophyll content  $(mg/g \text{ mass}) = 0.01 \times (20.21 \times A645 + 8.02 \times A663) \times F \div W = 0.01 \times (20.21 \times 0.362 + 8.02 \times 0.882) \div W = 1.439 \text{ mg/g}$ 

#### **Related Products:**

AK0250/AK0241 Glyceraldehyde-3-phosphate Dehydrogenase(GAPDH) Activity Assay Kit

AK0080/AK0079 Plant Carotenoid Content Assay Kit