



Plant Chlorophyll Content Assay Kit

Note: It is necessary to predict 2-3 large difference samples before the formal determination.

Operation Equipment: Spectrophotometer/ microplate reader

Catalog Number: AK0299

Size: 100T/96S

Product Composition: Before use, please carefully check whether the volume of the reagent is consistent with the volume in the bottle.

Reagent name	Size	Preservation Condition
Extract solution	Liquid×1(Required but not provided)	2-8℃
Reagent I	Powder×1	2-8℃

Solution Preparation:

1. Extract solution: Anhydrous ethanol and acetone are required but not provided. Mix anhydrous ethanol: acetone (V: V) = 1:2 for use. Provide a 125ml empty bottle.

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 was 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/microplate reader, micro glass cuvette/96 well plate (non-polystyrene 96 well plate is recommended), transferpettor, balance, mortar/homogenizer, silver paper, distilled water, 10mL tube, anhydrous ethanol (>98%, AR) and acetone (>98%, AR).

Operation procedure:

I. Sample preparation:(The sample size to be tested can be adjusted appropriately, and the specific proportion can be referred to the literature.)

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 Extract solution. Transfer all flushing solution into 10 mL EP tube. Use Extract solution 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 extraction until the color of tissue residue is close to white.

II. Determination procedure:

1. Preheat the spectrophotometer/microplate reader for more than 30 minutes, adjust the wavelength to 645 nm and 663 nm, set zero with Extract solution.
2. Take 200 μ L of the upper extract and put it into a micro glass cuvette/96 well plate. (if polystyrene 96 well plate is used, please complete the measurement as soon as possible within 5 min). Measure the absorbance value at 663 nm and 645 nm, recorded as A663 and A645, respectively.

III. Calculation:

$$\begin{aligned}\text{Chlorophyll a content (mg/g fresh mass)} &= (21.2 \times A_{663} - 4.48 \times A_{645}) \times VE \times F \div W \div 1000 \\ &= 0.01 \times (21.2 \times A_{663} - 4.48 \times A_{645}) \times F \div W\end{aligned}$$

$$\begin{aligned}\text{Chlorophyll b content (mg/g fresh mass)} &= (38.2 \times A_{645} - 7.8 \times A_{663}) \times VE \times F \div W \div 1000 \\ &= 0.01 \times (38.2 \times A_{645} - 7.8 \times A_{663}) \times F \div W\end{aligned}$$

$$\begin{aligned}\text{Total chlorophyll content (mg/g fresh mass)} &= (33.7 \times A_{645} + 13.4 \times A_{663}) \times VE \times F \div W \div 1000 \\ &= 0.01 \times (33.7 \times A_{645} + 13.4 \times A_{663}) \times F \div W\end{aligned}$$

VE: Extract solution volume, 10 mL;

W: Sample mass, 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 VE can be reduced properly. Pay attention to change the value of V extraction in the calculation formula.
5. If polystyrene 96 well plate is used for measurement, please complete the measurement as soon as possible within 5 min.

Experimental example:

1. 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, $A_{663} = 0.637$, $A_{645} = 0.27$.

Chlorophyll a content (mg/g mass) = $0.01 \times (21.2 \times A_{663} - 4.48 \times A_{645}) \times F \div W = 1.23$ mg/g mass.

Chlorophyll b content (mg/g mass) = $0.01 \times (38.2 \times A_{645} - 7.8 \times A_{663}) \times F \div W = 0.53$ mg/g mass.

Total chlorophyll content (mg/g mass) = $0.01 \times (33.7 \times A_{645} + 13.4 \times A_{663}) \times F \div W = 1.76$ mg/g mass.

Related Products:

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

KitAK0080/AK0079 Plant Carotenoid Content Assay Kit