

## Plant Chlorophyll Content Assay Kit

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

**Operation Equipment:** Spectrophotometer

**Catalog Number:** BC0990

**Size:** 50T/48S

**Product Composition:** Before use, please carefully check whether the volume of the reagent is consistent with the volume in the bottle. If you have any questions, please contact Solarbio staff in time.

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

### Solution Preparation:

1. Extract: 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 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, 10mL tube, anhydrous ethanol (>98%, AR) and acetone (>98%, AR).

### 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 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:

1. Preheat spectrophotometer for more than 30 minutes, adjust wavelength to 645 nm and 663 nm, set 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:

$$\begin{aligned} \text{Chlorophyll a content (mg/g weight)} &= (12.7 \times A_{663} - 2.69 \times A_{645}) \times V_E \times F \div W \div 1000 \\ &= 0.01 \times (12.7 \times A_{663} - 2.69 \times A_{645}) \times F \div W \end{aligned}$$

$$\begin{aligned} \text{Chlorophyll b content (mg/g weight)} &= (22.9 \times A_{645} - 4.68 \times A_{663}) \times V_E \times F \div W \div 1000 \\ &= 0.01 \times (22.9 \times A_{645} - 4.68 \times A_{663}) \times F \div W \end{aligned}$$

$$\begin{aligned} \text{Total chlorophyll content (mg/g weight)} &= (20.21 \times A_{645} + 8.02 \times A_{663}) \times V_E \times F \div W \div 1000 \\ &= 0.01 \times (20.21 \times A_{645} + 8.02 \times A_{663}) \times F \div W \end{aligned}$$

$V_E$ : 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:

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,  $A_{663} = 0.882$ ,  $A_{645} = 0.362$ .

$$\text{Chlorophyll a content (mg/g weight)} = 0.01 \times (12.7 \times A_{663} - 2.69 \times A_{645}) \times F \div W = 1.023 \text{ mg/g weight.}$$

$$\text{Chlorophyll b content (mg/g weight)} = 0.01 \times (22.9 \times A_{645} - 4.68 \times A_{663}) \times F \div W = 0.416 \text{ mg/g weight.}$$

$$\text{Total chlorophyll content (mg/g weight)} = 0.01 \times (20.21 \times A_{645} + 8.02 \times A_{663}) \times F \div W = 1.439 \text{ mg/g weight.}$$

#### Recent Product Citation:

[1] Su H, Zhang Y, Liu Y, Lu R, Gao A, Han Q, Wen B, Hu B, Yang P. Enhancing Bioavailability of Fertilizer through an Amyloid-Like Protein Coating. *Adv Mater.* 2023 Jul;35(30):e2300829. doi: 10.1002/adma.202300829. Epub 2023 Jun 6. PMID: 37074223.

[2] Xiao C, Sun D, Liu B, Fang X, Li P, Jiang Y, He M, Li J, Luan S, He K. Nitrate transporter NRT1.1 and anion channel SLAH3 form a functional unit to regulate nitrate-dependent alleviation of ammonium toxicity. *J Integr Plant Biol.* 2022 Apr;64(4):942-957. doi: 10.1111/jipb.13239. PMID: 35229477.

[3] Su L, Guo D, Wan H, Wang P, Cao L, Long Y, Chen C, Song Y, Zhang Y, Zeng C, Guo R, Liu X. Transcriptomic and metabolomic insights into the defense response to HFRs in Arabidopsis. *Ecotoxicol Environ Saf.* 2023 Apr 1;254:114736. doi: 10.1016/j.ecoenv.2023.114736. Epub 2023 Mar 9. PMID: 36905847.

[4] Jiang Y, Zhang H, Li Y, Chang C, Wang Y, Feng H, Li R. A Novel Transcriptional Regulator HbERF6 Regulates the HbCIPK2-Coordinated Pathway Conferring Salt Tolerance in Halophytic *Hordeum brevisubulatum*. *Front Plant Sci.* 2022 Jul 7;13:927253. doi: 10.3389/fpls.2022.927253. PMID: 35873960; PMCID: PMC9302439.

[5] Zhu Y, Wang Q, Wang Y, Xu Y, Li J, Zhao S, Wang D, Ma Z, Yan F, Liu Y. Combined Transcriptomic and Metabolomic Analysis Reveals the Role of Phenylpropanoid Biosynthesis Pathway in the Salt Tolerance Process of *Sophora alopecuroides*. *Int J Mol Sci.* 2021 Feb 27;22(5):2399. doi: 10.3390/ijms22052399. PMID: 33673678; PMCID: PMC7957753.

**Related Products:**

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|---------------|---|
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| BC4330/BC4335 | Plant Carotenoid Content Assay Kit                                  |