

(FOR RESEARCH USE ONLY. DO NOT USE IT IN CLINICAL DIAGNOSIS!)

Chlorophyll Assay Kit

Catalog No.: BC00188

Size: 50T

Please read the instructions carefully before use. If you have any questions or need further help during experiment, please don't hesitate to contact us through the following methods:

✉ Email (Sale)	order@enkilife.com
✉ Email (Techsupport)	techsupport@enkilife.com
☎ Tel:	0086-27-87002838
🌐 Website:	www.enkilife.com

Shelf life: Please refer to the label on the outer package.

Techsupport: In order to provide you with better service, please inform us the lot number on the label of the outer package.

Basic Information

Product Name	Chlorophyll Assay Kit
Detection Method	Colorimetric
Sample Type	Plant
Assay Type	Quantitative
Detection Instrument	Spectrophotometer (645 nm and 663 nm)

Principle

Chlorophyll a and chlorophyll b have maximum absorption peaks at 645 nm and 663 nm, respectively. Based on the absorption of visible spectrum by the chlorophyll extract, the absorbance is measured at the maximum absorption wavelengths using a spectrophotometer, so as to calculate the contents of chlorophyll a, chlorophyll b and total chlorophyll.

Components

No.	Components	Size	Note
Reagent 1	Powder	1 vial	Store at 4°C

Preparation of extract: Mix absolute ethanol and acetone thoroughly at a volume ratio of 1:2 (V: V) to prepare the extract for later use.

Storage

The kit has a validity period of 3 months.

Preparation

Visible spectrophotometer, 1 cm path-length glass cuvette, 10 mL centrifuge tube, mortar, balance, absolute ethanol, acetone and distilled water.

Operation process

Grinding with liquid nitrogen:

1. Take fresh plant leaves (remove the midrib) or other tissues, rinse thoroughly with distilled water, and blot the surface with absorbent paper. Grind the samples into powder with liquid nitrogen under dark conditions. Accurately weigh approximately 0.1 g of plant powder into a 10 mL centrifuge tube.
2. Add 1 mL of distilled water, then weigh and add about 50 mg of Reagent 1 powder, and mix well.
3. Adjust the volume to 5 mL with the extract, mix thoroughly, and react in the dark for more than 3 hours until the leaf powder turns completely white.
4. Centrifuge at 4000 rpm for 10 minutes. Collect the supernatant and dilute it to an appropriate concentration with the extract (OD value < 1). Zero the spectrophotometer with the extract, then measure and record the absorbance values at 645 nm and 663 nm respectively.

Mechanical homogenization method:

1. Take fresh plant leaves (with midribs removed) or other tissues, rinse with distilled water and blot dry with filter paper. Weigh approximately 0.1 g of shredded tissue and place it into a mortar.
2. Add 1 mL of distilled water and a small amount of Reagent 1 (50 mg). Fully grind the mixture under dark conditions, then transfer it into a 10 mL glass test tube.
3. Rinse the mortar with the extract solution and transfer all the rinsing liquid into the test tube. Make up the volume to 5 mL with the extract solution, and conduct extraction in the dark for no less than 3 h until the leaf powder turns completely white.
4. Centrifuge the mixture at 4000 rpm for 10 minutes. Collect the supernatant and dilute it to an appropriate concentration with the extract solution (OD value < 1). Zero the spectrophotometer using the extract solution, then measure and record the absorbance value A at 645 nm and 663 nm respectively.

Calculation

$$\text{Chlorophyll a content (mg/g)} = (12.7 \times A_{663} - 2.69 \times A_{645}) \times V_{\text{Extraction}} \times N \div m \div 1000$$

$$\text{Chlorophyll b content (mg/g)} = (22.9 \times A_{663} - 4.68 \times A_{645}) \times V_{\text{Extraction}} \times N \div m \div 1000$$

$$\text{Chlorophyll a content (mg/g)} = (20.21 \times A_{663} + 8.02 \times A_{645}) \times V_{\text{Extraction}} \times N \div m \div 1000$$

Annotation:

$V_{\text{Extraction}}$: Total volume of extraction solution, 5 mL

N: Dilution factor before colorimetry

m: Sample mass, g

Precautions

1. It is recommended to use disposable laboratory test tubes for operation.
2. This product is intended for scientific research use only by professionals and must not be used for clinical diagnosis or treatment, in food or drugs, or stored in ordinary residences.
3. Chlorophyll is light-sensitive; all operations should be performed under dark conditions as much as possible.
4. For sufficient extraction, fully extract the tissue residues until they turn completely white.
5. Rinse the mortar thoroughly with the extract solution, and transfer all green substances into the centrifuge tube.
6. If the absorbance value exceeds 1, appropriately dilute the supernatant.