

Chlorophyll GENLISA™ Assay

Cat No: KBCA1301

Pack: 50T/48S

1. Determination principle:

Chlorophyll a and chlorophyll b have maximum absorption peaks at 645 nm and 663 nm respectively. According to the absorption of the chlorophyll extract to the visible spectrum, the absorbance is measured at the maximum absorption wavelength using a spectrophotometer to calculate the chlorophyll a, chlorophyll b and total chlorophyll content.

2. Instruments and materials (self-provided):

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

3. Reagent composition: (50T/48 samples)

Reagent 1: Powder × 1 bottle, stored at 4°C;

Preparation of extraction solution: Mix anhydrous ethanol and acetone in a ratio of 1:2 (v:v) to prepare the extraction solution for later use.

4. Operation steps:

Method 1: (Liquid nitrogen grinding)

1. Take fresh plant leaves (remove the midrib) or other tissues, wash them with distilled water, dry the surface with absorbent paper, add liquid nitrogen and grind them into powder under dark conditions, and accurately weigh about 0.1 g of plant powder in a 10 mL centrifuge tube ;
2. Add 1 mL of distilled water, weigh about 50 mg of reagent powder and add it, mix well;
3. Use the extract to make up to 5 mL, mix well, and react for more than 3 hours in the dark (until the leaf powder turns white);
4. Centrifuge at 4000 rpm for 10 minutes, dilute the supernatant (with extraction solution) to an appropriate concentration (OD value < 1), adjust the extraction solution to zero, and read the absorbance values at 645 nm and 663 nm using a spectrophotometer.

Method 2: (Mechanical homogenization)

1. Take fresh plant leaves (remove the midrib) or other tissues, wash them with distilled water and dry them with absorbent paper, weigh about 0.1 g, cut them into pieces and put them in a mortar.
2. Add 1 mL of distilled water and a small amount of reagent 1 (50 mg), grind thoroughly in a dark environment, and place in a 10 mL glass test tube.
3. Rinse the mortar with the extract, transfer the wash solution to a glass test tube, make up to 5 mL of the extract, and extract for more than 3 hours in a dark environment (until the leaf powder turns completely white).
4. Centrifuge at 4000 rpm for 10 minutes, take the supernatant (with extraction solution) and dilute it to the appropriate concentration (OD value < 1), adjust the extraction solution to zero, and read the absorbance value A at 645 nm and 663 nm respectively.

5. Calculation formula:

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

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

Total chlorophyll content (mg/g fresh weight) = $(20.21 \times A_{645} + 8.02 \times A_{663}) \times V_{\text{extract}} \times N \div m \div 1000$

Note: V_{extract} : total volume of extract (5mL) ; N : dilution multiple before colorimetry; m : sample mass (g).

6. Notes:

1. Chlorophyll is sensitive to light, so all operations should be carried out in dark conditions as much as possible;
2. In order to fully extract, the tissue residue must be extracted until it turns completely white;
3. Rinse the mortar thoroughly with the extraction solution and transfer all the green matter to the centrifuge tube;
4. If the absorbance exceeds 1, the supernatant can be appropriately diluted.

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