

Chlorophyll Content Assay Kit

(Organic Solvent, Micro Method)

C1508199

Storage: Room Temperature.

Introduction:

Chloroplasts are organelles for photosynthesis. In photosynthesis research, extracted chloroplasts are often needed for downstream studies. The pigments contained in chloroplasts mainly fall into two categories: chlorophyll (including chlorophyll a and chlorophyll b) and carotenoids (including carotene and lutein). These pigments bind to proteins on the thylakoid membrane to form pigment-protein complexes, among which chlorophyll is also known as chloroplast pigments (Chlorophyll).

Detection Principle:

Chlorophyll is insoluble in water but soluble in organic solvents. Chlorophyll is extracted using an organic solvent. According to the Lambert-Beer law, the absorbance (A) of a colored solution is proportional to its solute concentration (C) and path length (L), i.e., $A = \alpha CL$, where α is the proportionality constant. When the solution concentration is expressed in percentage concentration and the path length is 1 cm, α is the absorption coefficient of the substance. In this kit, chlorophyll a and chlorophyll b have maximum absorption peaks at 665 nm and 649 nm, respectively. The contents of chlorophyll a, chlorophyll b, and total chlorophyll can be calculated using empirical formulas. This kit is primarily used for the extraction of chlorophyll from plant tissues and the quantitative detection of chlorophyll a, chlorophyll b, and total chlorophyll content.

Applicable Sample: Plants:

C1508199	Component	50T	100T	Storage
C1508199A	Chlorophyll Assay Buffer	530mL	530mL×2	RT.
C1508199B	Extraction Powder	3 g	6g	RT.

Reagents, consumables and Equipments not provided:

- Microplate reader (capable of measuring absorbance at 665 nm and 649 nm).
- Mortar or homogenizer, Centrifuge.
- 96-well plate, Centrifuge tubes, Filter paper or gauze.

Procedure:

- 1 Chloroplast Extraction:

- 1.1 Take fresh spinach leaves or other plant leaves, wash, dry, remove the midrib, weigh 0.1 g of the cut sample, place it in a mortar or homogenizer, add a small amount of Extraction Powder (about 50 mg) and 1 mL of Chlorophyll Assay Buffer, and grind or homogenize into a liquid state.
- 1.2 Transfer the ground or homogenized liquid to a 10 mL centrifuge tube. Rinse the mortar or homogenizer several times with a small amount of Chlorophyll Assay Buffer. Finally, pour everything, including the residue, into the 10 mL centrifuge tube. Add Chlorophyll Assay Buffer to a total volume of 10 mL, mix well, and incubate protected from light for 5 minutes to 2 hours.

*Note: Alternatively, the tissue can be cut into pieces, Chlorophyll Assay Buffer added, and incubated protected from light for 12-36 hours, shaking several times during this period to ensure full contact between the tissue and the extraction reagent. When the tissue turns nearly white, it indicates that pigment extraction is complete. If a significant amount of tissue color remains, continue the extraction until the tissue residue color is close to white.

- 2 Filter through filter paper or three layers of gauze, and collect the filtrate. Alternatively, centrifuge at 4,000 rpm for 5 minutes, and collect the supernatant for testing.

3 Measurement:

Turn on and preheat the microplate reader for at least 30 minutes. Set multiple wavelengths to 665 nm and 649 nm. Add 200 μ L of the chlorophyll extract to a 96-well plate. Zero the instrument with Chlorophyll Assay Buffer. Measure the absorbance of the extract at 665 nm and 649 nm (A_{665} and A_{649}) using the microplate reader.

4 Calculation of Results:

Chlorophyll a content (mg/g) = $C_a \times V \times N / (W \times 1000)$

Chlorophyll b content (mg/g) = $C_b \times V \times N / (W \times 1000)$

Total chlorophyll content (mg/g) = $C_T \times V \times N / (W \times 1000)$

Parameter Description:

$C_a = (13.95 \times A_{665} - 6.88 \times A_{649}) / 0.6 = 23.25 \times A_{665} - 11.467 \times A_{649}$ (mg/L)

$C_b = (24.96 \times A_{649} - 7.32 \times A_{665}) / 0.6 = 41.6 \times A_{649} - 12.2 \times A_{665}$ (mg/L)

$C_T = (6.63 \times A_{665} + 18.08 \times A_{649}) / 0.6 = 11.05 \times A_{665} + 30.133 \times A_{649}$ (mg/L)

V: Volume of chlorophyll extract (mL) = 10 (mL).

N: Dilution factor.

W: Fresh or dry weight of the sample (g).

1000: Unit conversion factor between mL and L.

0.6: Light path length (cm) for 200 μ L extract in the microplate.

Notes:

1. To avoid photodegradation of chlorophyll, perform operations under minimal light and keep grinding or homogenization time as short as possible.
2. If the sample absorbance is greater than 1, dilute appropriately before measurement and multiply by the dilution factor in the calculation.
3. Chlorophyll Assay Buffer is volatile; always tighten the cap when not in use.

4. The chlorophyll extract must not appear turbid; if turbidity occurs, filter again.
5. For your safety and health, please wear a lab coat and disposable gloves during operation.
6. Use reagents as soon as possible after opening to avoid affecting subsequent experimental results.
7. This kit is intended for research use only and is not suitable for clinical diagnosis or other purposes.

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