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CheKine™ Micro Plant Chlorophyll Content Assay Kit

Cat #: KTB3022 Size: 96 T/96 S

	Micro Plant Chlorophyll Content Assay Kit		
REF	**Cat #:** KTB3022	LOT	**Lot #:** Refer to product label
	Applicable sample: Plant Tissues		
	Storage: Stored at 4℃ for 6 months		

Assay Principle

Plant chlorophyll is widely present in green plant tissues, and its content is closely related to photosynthesis and nutritional status, which is an important indicator of plant growth status. Chlorophyll a and chlorophyll b have maximum absorption peaks at 645 nm and 663 nm. Based on empirical formulas, chlorophyll a, chlorophyll b, and total chlorophyll content can be calculated.

Materials Supplied and Storage Conditions

Kit components	Size (96 T)	Storage conditions
Reagent Ⅰ	Powder×1 vial (6 g)	4℃

Note: Before formal testing, it is recommended to select 2-3 samples with large expected differences for pre-experiment.

Materials Required but Not Supplied

- Microplate reader or visible spectrophotometer capable of measuring absorbance at 645 nm and 663 nm
- 96-well plate (non polystyrene material) or microglass cuvette, precision pipettes, disposable pipette tips
- Analytical balance
- Deionized water, anhydrous ethanol, acetone
- Mortar or homogenizer, 10 mL glass test tube, tin foil paper

Reagent Preparation

Extraction Buffer: Prepared before use. Prepare anhydrous ethanol and acetone by yourself, and mix anhydrous ethanol and acetone in a ratio of 1:2 by volume for use. Store at 4℃.

Reagent Ⅰ: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃.

Note: Extraction Buffer has a pungent odor, so it is recommended to experiment in a fume hood. Reagent Ⅰ has slight irritation, so personal protection is recommended during use.

Sample Preparation

Note: We recommend that you use fresh samples. If not assayed immediately, samples can be stored at -80℃ for one month. When measuring, the temperature and time of thawing should be controlled. When thawing at room temperature, the sample should be thawed within 4 h.

1. Take fresh plant leaves or other green tissues, wash them thoroughly with deionized water, then absorb surface moisture, remove the midrib, weigh about 0.1 g, cut them into small pieces, and place them in a mortar or homogenizer.
2. Add 1 mL of deionized water to the mortar, then add 50 mg Reagent Ⅰ (no dissolution required), grind thoroughly in dark or low light conditions, and transfer to a 10 mL glass test tube.
3. Rinse the mortar or homogenizer with an Extraction Buffer, transfer all rinse solution and green substances into a 10 mL glass test tube, dilute to 10 mL with Extraction Buffer, and immerse in dark conditions or wrap tin foil paper for 3 h. Observe that the bottom tissue residue color is close to white, then extract completely. If the tissue residue does not completely turn white, continue to extract until the tissue residue color is close to white.

Assay Procedure

1. Preheat the microplate reader or visible spectrophotometer for more than 30 min, and adjust the wavelength to 645 nm and 663 nm, visible spectrophotometer was returned to zero with Extraction Buffer.
2. Take the upper extraction solution 200 μL and measure the absorbance values at 663 nm and 645 nm in microglass cuvette or 96 well plate (if using a polystyrene 96 well plate, please complete the measurement as soon as possible within 5 min), and record them as A_{663} and A_{645} respectively.

Note: Before the experiment, it is suggested that 2-3 samples with large expected differences should be selected for pre-experiment. If absorbance is less than 0.1, increase the sample quantity appropriately. If absorbance values is greater than 1.0, the sample can be appropriately diluted with Extraction Buffer, the calculated result multiplied by the dilution factor, or decrease the sample quantity appropriately.

Data Analysis

Note: We provide you with calculation formulae, including the derivation process and final formula. The two are exactly equal. It is suggested that the concise calculation formula in bold is final formula.

Chlorophyll a content (mg/g fresh weight)=$(12.7\times A_{663}-2.69\times A_{645})\times V_{Total\ Sample}\times F\div W\div 1{,}000$**$=0.01\times(12.7\times A_{663}-2.69\times A_{645})\times F\div W$**

Chlorophyll b content (mg/g fresh weight)=$(22.9\times A_{645}-4.68\times A_{663})\times V_{Total\ Sample}\times F\div W\div 1{,}000$**$=0.01\times(22.9\times A_{645}-4.68\times A_{663})\times F\div W$**

Total chlorophyll content (mg/g fresh weight)=$(12.7\times A_{663}-2.69\times A_{645})\times V_{Total\ Sample}\times F\div W\div 1{,}000+(22.9\times A_{645}-4.68\times A_{663})\times V_{Total\ Sample}\times F\div W\div 1{,}000=(20.21\times A_{645}+8.02\times A_{663})\times V_{Total\ Sample}\times F\div W\div 1{,}000$**$=0.01\times(20.21\times A_{645}+8.02\times A_{663})\times F\div W$**

$V_{Total\ Sample}$: Extraction Buffer volume, 10 mL; F: dilution factor; W: sample weight, g.

Precautions

1. Chlorophyll is sensitive to light, and grinding and extraction operations should be avoided from light or carried out under low light conditions.
2. Different blades have different hardness, so try to grind them thoroughly.
3. Rinse the mortar with the extraction solution until all green substances are transferred to the glass test tube.
4. It is necessary to extract until the tissue residue completely turns white, otherwise the extraction is insufficient.

Typical Data

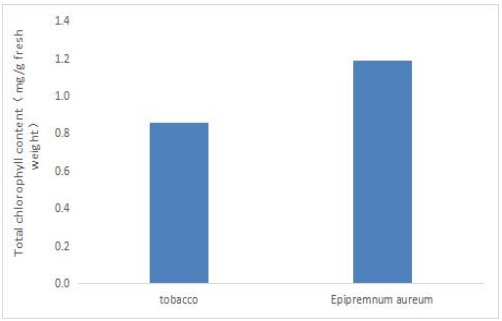

Figure 1. Determination total chlorophyll content in tobacco and epipremnum aureum leaves by this assay kit

Recommended Products

Catalog No.	Product Name
KTB1100	CheKine™ Micro Lactic Acid (LA) Assay Kit
KTB1121	CheKine™ Pyruvate Acid (PA) Colorimetric Assay Kit

Disclaimer

The reagent is only used in the field of scientific research, not suitable for clinical diagnosis or other purposes. For your safety and health, please wear a lab coat and disposable gloves.