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CheKine™ Mirco Carotenoid Content Assay Kit

Cat #: KTB3026 Size: 48 T/48 S 96 T/96 S

	Mirco Carotenoid Content Assay Kit		
REF	**Cat #:** KTB3026	LOT	**Lot #:** Refer to product label
	Applicable sample: Plant Tissues		
	Storage: Stored at 4℃ for 6 months, protected from light		

Assay Principle

Carotenoids are lipid-soluble compounds with nutritional properties that provide natural pigments to plants and animals. They are important antioxidants and have the ability to convert into essential vitamins. Carotenoids can prevent damage to cells, tissues, and genes, enhance the body's immune system, resist infections, reduce the risk of cancer, and protect the heart. CheKine™ Mirco Carotenoid Content Assay Kit provides a simple, convenient, and rapid method for measuring carotenoid content, suitable for plant tissue samples. The principle involves carotenoids in plants are found in various yellow plastids or chromoplasts, such as in yellow leaves, yellow flowers, yellow and red fruits, and yellow roots. Samples are extracted using solvents to separate and extract carotenoids. Carotenoids have a specific absorption peak at 440±10 nm. In higher plants and algal microorganisms, chloroplasts also contain carotenoids. Carotenoids primarily absorb blue and violet light, while chlorophyll a and b absorb both red and blue-violet light. Therefore, for tissues containing chloroplasts, to exclude the interference from chlorophyll a and b on carotenoids, the content of chlorophyll a and b is first calculated using empirical formulas. Then, the carotenoid content is determined. For tissues without chlorophyll, the carotenoid content can be directly calculated using the empirical extinction coefficient of carotenoids.

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	48 T	96 T	
Extraction Buffer	Self-provided	Self-provided	4℃, protected from light
Reagent I	Powder×1 vial	Powder×1 vial	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 440/470/646/663 nm
- 96-well plate (non-polystyrene material) or microglass cuvette, precision pipettes, disposable pipette tips, 10 mL centrifuge tube/test tube
- Analytical balance, centrifuge

- Deionized water, acetone
- Homogenizer

Reagent Preparation

Extraction Buffer: Prepare 80% acetone by mixing acetone: deionized water (V:V)=4:1. Provide a 125 mL empty bottle for the mixture. Store at 4℃, protected from light.

Note: Acetone has a strong, irritating odor. It is recommended to take appropriate protective measures and perform the operation in a fume hood.

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

Sample Preparation

Note: We recommend that you use fresh samples. If not assayed immediately, samples can be stored at -80℃ for up to 2 weeks. 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. Fresh plant leaves (with the midrib removed) or other tissues should be washed clean with deionized water, then blotted dry to remove surface moisture. Weigh approximately 0.1 g of the tissue, cut it into small pieces, and place it in a mortar or homogenizer.
2. Add 1 mL of deionized water and a small amount of Reagent I (approximately 10 mg). Grind thoroughly in the dark or under low light conditions. Transfer the mixture to a 10 mL centrifuge tube or test tube.
3. Rinse the mortar or homogenizer with Extraction Buffer and transfer all the rinse solution to the 10 mL centrifuge tube or test tube. Adjust the final volume to 10 mL with Extraction Buffer. Incubate in the dark or wrap the tube with aluminum foil for 3 h (invert and mix the tube twice during this period). Observe the bottom of the tube; if the tissue residue is nearly white, the extraction is complete. If the tissue residue is not completely white, continue the extraction until the residue color approaches white.

Assay Procedure

A. Carotenoid Content Measurement for Yellow or Other Non-Green Tissues (Without Chloroplasts)

1. Preheat the microplate reader or visible spectrophotometer for 30 min and set the wavelength to 440 nm. Zero the visible spectrophotometer using Extraction Buffer.
2. Sample Measurement (Using microglass cuvette: No blank tube measurement is required. Using 96-well Plate (Non-Polystyrene Material): Measure the blank tube only 1-2 times.)

Reagent	Test Well (μL)	Blank Well (μL)
Sample	200	0
Extraction Buffer	0	200

Using a Spectrophotometer: quickly measure the absorbance at 440 nm in a micro glass cuvette and record it as A_{440}.Using a Microplate Reader: In a 96-well plate (non-polystyrene material), quickly measure the absorbance at 440 nm. Record the absorbance of the Test well as $A_{440\ Test}$. Record the absorbance of the Blank well as $A_{440\ Blank}$. Calculate $\Delta A440=A_{440\ Test}-A_{440\ Blank}$.

B. Steps for Determining Carotenoid Content in Fresh Plant Leaves or Other Green Tissues (Containing Chloroplasts)

1. Preheat the microplate reader or visible spectrophotometer for 30 min and set the wavelength to 470 nm, 646 nm and 663 nm. Zero the visible spectrophotometer using Extraction Buffer.
2. Sample Measurement (Using microglass cuvette: No blank tube measurement is required. Using 96-well Plate (Non-Polystyrene Material): Measure the blank tube only 1-2 times.)

Reagent	Test Well (μL)	Blank Well (μL)
Sample	200	0
Extraction Buffer	0	200

Using a Spectrophotometer: quickly measure the absorbance at 470 nm, 646 nm, and 663 nm in a micro glass cuvette. Record these values as A_{470}, A_{646}, and A_{663}, respectively.Using a Microplate Reader: In a 96-well plate (non-polystyrene material), quickly measure the absorbance at 470 nm, 646 nm, and 663 nm. Record the absorbance of the Test wells as $A_{470\ Test}$, $A_{646\ Test}$, and $A_{663\ Test}$. Record the absorbance of the blank wells as $A_{470\ Blank}$, $A_{646\ Blank}$, and $A_{663\ Blank}$. Calculate: $\Delta A_{470}=A_{470\ Test}-A_{470\ Blank}$, $\Delta A_{646}=A_{646\ Test}-A_{646\ Blank}$, $\Delta A_{663}=A_{663\ Test}-A_{663\ Blank}$.

Note: If there are residues in the upper layer of the extraction solution: Transfer 1 mL of the upper layer of the extraction solution to a 1.5 mL brown EP tube. Centrifuge at 4,000 r/min for 5 min at room temperature. Then, take the supernatant for measurement. If using a polystyrene 96-well plate: Complete the measurement as soon as possible within 5 min after adding the samples. If A is less than 0.1, the sample volume can be appropriately increased. If A is greater than 1.0, the sample can be further diluted with Extraction Buffer before proceeding with the experiment, and the final dilution factor should be taken into account in the calculations.

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.

Calculation of Carotenoid Content:

A. Calculation Formula for Carotenoid Content in Yellow or Other Non-Green Tissues (Without Chloroplasts):

1. 96-well plate (non-polystyrene material) calculation formula as below

Carotenoid content (mg/g mass)=$A_{440}\div(\varepsilon\times d)\times V_{Total\ Sample}\times 1{,}000\div W\times F$**$=0.08\times A_{440}\times F\div W$**

Where: $V_{Total\ Sample}$: Total volume of Extraction Buffer, 0.01 L; 1,000: Conversion factor, 1 g=1,000 mg; ε: Empirical extinction coefficient for carotenoids, 250 L/g/cm; d: Path length of the 96-well plate (non-polystyrene material), 0.5 cm; F: Dilution factor; W: Sample mass, g.

2. Microglass cuvette calculation formula:

The optical diameter d: 0.5 cm in the above calculation formula can be adjusted to d: 1 cm for calculation.

B. Calculation Formula for Carotenoid Content in Fresh Plant Leaves or Other Green Tissues (Containing Chloroplasts)

1. 96-well plate (non-polystyrene material) calculation formula as below

C_a(mg/L)=$(12.21\times\Delta A_{663}-2.81\times\Delta A_{646})\div 0.5$**$=24.42\times\Delta A_{663}-5.62\times\Delta A_{646}$**

C_b(mg/L)=$(20.13\times\Delta A_{646}-5.03\times\Delta A_{663})\div 0.5$**$=40.26\times\Delta A_{646}-10.06\times\Delta A_{663}$**

Carotenoid Concentration: C_c(mg/L)=$(1{,}000\times\Delta A_{470}\div 0.5-3.27\times C_a-104\times C_b)\div 229$**$=8.734\times\Delta A_{470}-0.014\times C_a-0.454\times C_b$**

Carotenoid Content (mg/g mass)=$C_c\times V_{Total\ Sample}\times F\div W$**$=0.01\times C_c\times F\div W$**

Where: $V_{Total\ Sample}$: Total volume of Extraction Buffer, 0.01 L, F: Dilution factor; W: Sample mass, g.

2. Microglass cuvette calculation formula:

The optical diameter d: 0.5 cm in the above calculation formula can be adjusted to d: 1 cm for calculation.

Precautions

1. If you are unsure whether the tissue contains chlorophyll, you can scan the sample extract using a spectrophotometer at wavelengths from 400 to 700 nm. Check for peaks between 640 and 670 nm; the presence of peaks indicates the presence of chlorophyll, while the absence of peaks indicates no chlorophyll.
2. If using a polystyrene 96-well plate, complete the measurement as soon as possible within 5 min after adding the samples.
3. To prevent the degradation of pigments due to light exposure, operations should be conducted in the dark or with minimal light exposure. The grinding or homogenization time should be kept as short as possible.
4. The extraction buffer is volatile and has an irritating odor, so appropriate protective measures should be taken during handling.

Typical Data

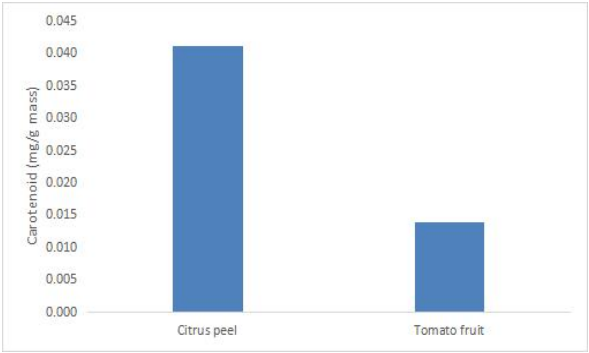

Figure 1. Determination carotenoid activity in Citrus peel and Tomato fruit by this assay kit

Recommended Products

Catalog No.	Product Name
KTB1015	CheKine™ Micro α-Glucosidase Activity 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.