Carotenoid Content Assay Kit - Micro Method

Product Information

Product Code	112560
Assay Size	100T
Method	Micro method

Product Introduction

Carotenoids are an important class of natural pigments widely found as yellow, orange-red, or red pigments in animals, higher plants, fungi, and algae.

Carotenoids are precursors of vitamin A in the body and are associated with antioxidant activity, immune regulation, anticancer effects, cardiovascular disease reduction, and use as colorants. In plants, carotenoids are present in xanthoplasts or chromoplasts in tissues such as yellow leaves, yellow flowers, yellow and red fruits, and yellow tuberous roots.

Samples are extracted with solvent, and carotenoids are separated and measured. Carotenoids show a characteristic absorption peak at 440±10 nm.

Most higher plants and algal microorganisms also contain carotenoids in chloroplasts. Carotenoids mainly absorb blue-violet light, while chlorophyll a and chlorophyll b absorb both red light and blue-violet light. For tissues containing chloroplasts, chlorophyll a and chlorophyll b are calculated first using empirical formulas to eliminate interference, and carotenoid content is then calculated. For tissues without chlorophyll, carotenoid content can be calculated directly using the empirical extinction coefficient of carotenoids.

Reference Measurement Example

Sample	Corn kernels
OD440 nm, blank	0.047
OD440 nm, assay	0.072 / 0.073 / 0.073

Actual readings may vary depending on the testing instrument and testing conditions. The data are for reference only.

Package Contents

Item Code	Component	Quantity
112560.1	Reagent I	2 g
112560.2	Extraction Solution	1 bottle, self-prepared
112560.m	Manual	1 copy

Extraction solution: prepare 80% acetone by mixing acetone and distilled water at a volume ratio of 4:1. One 125 mL empty bottle is provided.

Quality and Safety Information

Raw Material or Packaging Name	Quality Standard	Main Toxicity
Reagent I	--	--

Extraction Solution	--	--

Shipping and Storage

Shipping	Shipped with ice packs
Storage	Store at 2-8°C
Shelf Life	180 days

Instructions for Use

1. Sample Preparation

1. Wash fresh plant leaves with the midrib removed, or other tissues, with distilled water. Blot dry, weigh approximately 0.1 g, cut into pieces, and place in a mortar or homogenizer.
2. Add 1 mL distilled water and a small amount of Reagent I, approximately 10 mg. Grind thoroughly in the dark or under dim light, then transfer to a 10 mL centrifuge tube or test tube.
3. Rinse the mortar or homogenizer with extraction solution. Transfer all rinse solution to the 10 mL centrifuge tube or test tube, then bring the volume to 10 mL with extraction solution.
4. Keep the tube in the dark or wrap it with aluminum foil and extract for 3 h. During extraction, the tube may be inverted and mixed 2 times.
5. Extraction is complete when the tissue residue at the bottom appears nearly white. If the tissue residue has not turned nearly white, continue extraction until the color is close to white.

2. Measurement Steps for Yellow or Other Non-Green Tissues Without Chloroplasts

1. Preheat the spectrophotometer or microplate reader for at least 30 min. Set the wavelength to 440 nm and zero with extraction solution.
2. Transfer 200 μL of the supernatant extract into a micro quartz cuvette or 96-well plate.
3. Measure the absorbance at 440 nm and record it as A440.

3. Measurement Steps for Fresh Plant Leaves or Other Green Tissues With Chloroplasts

1. Preheat the spectrophotometer or microplate reader for at least 30 min. Set the wavelengths to 470 nm, 646 nm, and 663 nm, and zero with extraction solution.
2. Transfer 200 μL of the upper extract into a micro glass cuvette or 96-well plate.
3. Measure absorbance at 470 nm, 646 nm, and 663 nm. Record the values as A470, A646, and A663, respectively.

> If residue is present in the upper extract, pipette 0.3 mL of the upper extract into a 1.5 mL brown EP tube. Centrifuge at room temperature at 4000 r/min for 5 min, then use the supernatant for testing.

Calculation of Carotenoid Content

1. Yellow or Other Non-Green Tissues Without Chloroplasts

Micro Glass Cuvette Measurement

Carotenoid content (mg/g, mass) = A440 ÷ (ε × d) × V total sample × 1000 ÷ W × F = 0.04 × A440 × F ÷ W

- V total sample: total volume of extraction solution, 0.01 L
- 1000: unit conversion factor, 1 g = 1000 mg
- ε: empirical extinction coefficient of carotenoids, 250 L/g·cm-1
- d: cuvette optical path length, 1 cm
- F: dilution factor
- W: sample mass, g

96-Well Plate Measurement

Carotenoid content (mg/g, mass) = A440 ÷ (ε × d) × V sample total × 1000 ÷ W × F = 0.067 × A440 × F ÷ W

- V sample total: total volume of extract, 0.01 L
- 1000: unit conversion factor, 1 g = 1000 mg
- ε: empirical extinction coefficient of carotenoids, 250 L/g·cm-1
- d: 96-well plate optical path length, 0.6 cm
- F: dilution factor
- W: sample mass, g

2. Fresh Plant Leaves or Other Green Tissues Containing Chloroplasts

Micro Glass Cuvette Measurement

Ca (mg/L) = 12.21 × A663 - 2.81 × A646

Cb (mg/L) = 20.13 × A646 - 5.03 × A663

Carotenoid concentration: Cc (mg/L) = (1000 × A470 - 3.27 × Ca - 104 × Cb) ÷ 229 = 4.367 × A470 - 0.014 × Ca - 0.454 × Cb

Carotenoid content (mg/g, mass) = Cc × V extraction × F ÷ W = 0.01 × Cc × F ÷ W

- V extraction: extract volume, 0.01 L
- F: dilution factor
- W: sample mass, g

96-Well Plate Measurement

Ca (mg/L) = (12.21 × A663 - 2.81 × A646) ÷ 0.6 = 20.35 × A663 - 4.83 × A646

Cb (mg/L) = (20.13 × A646 - 5.03 × A663) ÷ 0.6 = 33.55 × A646 - 8.38 × A663

Carotenoid concentration: Cc (mg/L) = (1000 × A470 ÷ 0.6 - 3.27 × Ca - 104 × Cb) ÷ 229 = 7.278 × A470 - 0.014 × Ca - 0.454 × Cb

Carotenoid content (mg/g, mass) = Cc × V extraction × F ÷ W = 0.01 × Cc × F ÷ W

- V extraction: extract volume, 0.01 L
- F: dilution factor
- W: sample mass, g
- 0.6: optical path length ratio, 96-well plate optical path length to cuvette optical path length

Precautions

1. This 100T product can test 96 samples.
2. Prepare the following instruments and supplies separately: visible-light spectrophotometer, micro glass colorimetric cuvette or 96-well plate, adjustable pipette, balance, mortar or homogenizer, aluminum foil, 10 mL test tubes, acetone, and distilled water.
3. If it is uncertain whether chlorophyll in the tissue affects the result, scan the sample extract with a spectrophotometer at 400-700 nm. A peak between 640-670 nm indicates chlorophyll is present; if no peak is observed, chlorophyll is absent.
4. When A exceeds 1, dilute the sample with extraction solution before measurement and multiply by the dilution factor F in the formula.
5. To avoid pigment decomposition caused by light exposure, avoid light as much as possible during operation and keep the grinding or homogenization time as short as possible.
6. The extraction solution is volatile. Take protective measures during operation.
7. When measuring many samples, monitor the liquid level of the extraction solution in the cuvette used for zero calibration to prevent errors caused by evaporation.
8. Because acetone is corrosive, if using a polystyrene 96-well plate, complete the measurement as soon as possible within 5 min.