Carotenoid Content Assay Kit - Microplate Method

Product Information

Product Code	112561
Product Name	Carotenoid Content Assay Kit - Microplate Method
Format	100T
Sample Capacity	Up to 96 samples

Product Introduction

Carotenoids are an important class of natural pigments widely found in yellow, orange-red, and red pigments of animals, higher plants, fungi, and algae. In the body, carotenoids are precursors of vitamin A and may also provide antioxidant effects, immune regulation, anticancer effects, support for cardiovascular health, and coloring effects.

In plants, carotenoids are present in chromatophores or chromoplasts, such as yellow leaves, yellow flowers, yellow and red fruits, and yellow storage roots. Samples are extracted by solvent extraction to separate carotenoids, which have 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 contents are calculated first to eliminate their interference, then carotenoid content is calculated. For tissues without chlorophyll, carotenoid content can be calculated directly using the empirical extinction coefficient of carotenoids.

Example test result using corn kernels: OD440nm blank: 0.047; assay: 0.072 / 0.073 / 0.073. Actual readings may vary depending on the instrument and testing conditions; these values are for reference only.

Package Contents

Item Code	Component	Quantity
112561.1	Reagent I	2 g
112561.2	Extraction solution	Prepare before use; one 125 mL empty bottle provided
112561.m	Instructions	1 copy

Extraction Solution Preparation

Prepare 80% acetone by mixing acetone and distilled water at a volume ratio of 4:1. Prepare fresh before use.

Quality and Safety Information

Material or Package Name	Quality Standard	Main Toxicity
Reagent I	--	--
Extraction solution	--	--

Transportation and Storage

Condition	Requirement
Transportation	Transport with ice packs.
Storage	Store at 2-8°C.
Shelf Life	180 days.

Required Instruments and Supplies

- Microplate reader
- 96-well plate
- Adjustable pipette
- Balance
- Mortar or homogenizer
- Aluminum foil
- 10 mL test tubes
- Acetone
- Distilled water

Instructions for Use

1. Sample Processing

1. Wash fresh plant leaves, with the midrib removed, or other tissues thoroughly with distilled water. Absorb surface moisture, 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 under dark or low-light conditions, then transfer to a 10 mL centrifuge tube or test tube.
3. Rinse the mortar or homogenizer with extraction solution and transfer all rinse liquid to the 10 mL centrifuge tube or test tube. Bring the volume to 10 mL with extraction solution.
4. Place the tube under dark conditions or wrap it with aluminum foil. Extract for 3 h, inverting and mixing 2 times during extraction.
5. Observe the tissue residue at the bottom. If it is close to white, extraction is complete. If the residue has not turned close to white, continue extraction until the color is close to white.

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

1. Preheat the microplate reader for at least 30 min.
2. Set the wavelength to 440 nm and zero the instrument with extraction solution.
3. Add 200 μL of the upper-layer extract to a 96-well plate.
4. Measure absorbance at 440 nm and record the value as A440.

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

1. Preheat the microplate reader for at least 30 min.
2. Set the wavelengths to 470 nm, 646 nm, and 663 nm, and zero the instrument with extraction solution.
3. Add 200 μL of the upper-layer extract to a 96-well plate.
4. Measure absorbance at 470 nm, 646 nm, and 663 nm. Record the values as A470, A646, and A663.

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

Calculations

Yellow or Other Non-Green Tissues Without Chloroplasts

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

Symbol	Definition
Vtotal 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	96-well plate optical path length, 0.6 cm
F	Dilution factor
W	Sample mass, g

Fresh Plant Leaves or Other Green Tissues Containing Chloroplasts

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 × Vextraction × F ÷ W = 0.01 × Cc × F ÷ W

Symbol	Definition
Vextraction	Extract volume, 0.01 L
F	Dilution factor
W	Sample mass, g
0.6	Optical path ratio: 96-well plate optical path to cuvette optical path

Precautions

1. This 100T kit can test 96 samples.
2. If it is uncertain whether chlorophyll in the tissue affects the result, scan the sample extract with a spectrophotometer at 400-700 nm and check for an absorption peak at 640-670 nm. If a peak is present, chlorophyll is present; otherwise, chlorophyll is absent.
3. When A exceeds 1, dilute the sample with extraction solution before measurement and multiply by the dilution factor F in the calculation formula.
4. To avoid pigment decomposition under light, protect samples from light as much as possible during operation. Minimize grinding or homogenization time.
5. The extraction solution is volatile; take protective measures during operation.
6. When measuring many samples, monitor the liquid level of the extraction solution used for zero calibration to prevent errors caused by volatilization.
7. Acetone is corrosive. If using a polystyrene 96-well plate, complete the measurement as soon as possible within 5 min.