Carotenoid Content Assay Kit - Spectrophotometric Method

Product Information

Product Code	112558
Assay Size	50T
Method	Spectrophotometric method

Product Introduction

Carotenoids are an important class of natural pigments widely found in animals, higher plants, fungi, and algae. They are responsible for many yellow, orange-red, and red colors.

Carotenoids are precursors of vitamin A in the body and also have antioxidant, immunomodulatory, anticancer, cardiovascular disease-reducing, and coloring functions.

In plants, carotenoids occur in xanthoplasts or chromoplasts, such as in yellow leaves, yellow flowers, yellow and red fruits, and yellow tuber tissues. Samples are extracted with solvent, and carotenoids are separated and measured by their 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 first calculated using empirical formulas to correct their interference, and then carotenoid content is calculated. For tissues without chlorophyll, carotenoid content can be calculated directly using the empirical extinction coefficient.

> Example measurement using corn kernels: OD440nm readings were 0.017, 0.015, and 0.016. Actual readings may vary depending on the testing instrument and test conditions. These data are for reference only.

Package Contents

Item Code	Component	Quantity
112558.1	Reagent I	1 g
112558.2	Extraction solution bottle	1 empty 125 mL bottle
112558.m	Instruction manual	1 copy

Extraction solution is self-prepared 80% acetone, made by mixing acetone and distilled water at a volume ratio of 4:1.

Quality and Safety Information

Component	Quality Standard	Main Toxicity
Reagent I	Not specified	Not specified
Extraction solution	Not specified	Not specified

Transport and Storage

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

Instructions

1. Sample Preparation

1. Wash fresh plant leaves, with the midrib removed, or other tissues with distilled water. Blot the surface dry, weigh about 0.1 g, cut into pieces, and place in a mortar or homogenizer.
2. Under dark or low-light conditions, add 1 mL distilled water and a small amount of Reagent I, about 10 mg. Grind thoroughly, then transfer to a 10 mL centrifuge tube or test tube.
3. Rinse the mortar or homogenizer with extraction solution. Transfer all rinses into the 10 mL centrifuge tube or test tube, then make up to 10 mL with extraction solution.
4. Place the tube in the dark or wrap it with aluminum foil and extract for 3 h. During extraction, invert and mix 2 times if needed.
5. Observe the tissue residue at the bottom. If it is nearly white, extraction is complete. If the residue has not turned nearly white, continue extraction until the residue is nearly white.

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

1. Preheat the spectrophotometer for 30 min or more.
2. Set the wavelength to 440 nm and zero with the extraction solution.
3. Transfer 1 mL of the supernatant extract into a 1 mL glass cuvette.
4. 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 for 30 min or more.
2. Set the wavelengths to 470 nm, 646 nm, and 663 nm, and zero with the extract.
3. Transfer 1 mL of the upper extract into a 1 mL glass cuvette.
4. Measure the absorbance at 470 nm, 646 nm, and 663 nm. Record the values as A470, A646, and A663.

> If the upper extract contains residue, pipette 1.2 mL of the upper extract into a 1.5 mL brown tube. Centrifuge at room temperature at 4000 r/min for 5 min, then take the supernatant for testing.

Calculations

1. Yellow or Other Non-Green Tissues Without Chloroplasts

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

Carotenoid content (mg/g, mass) = 0.04 × A440 × F ÷ W

Symbol	Meaning
Vtotal sample	Total extract volume, 0.01 L
1000	Unit conversion factor, 1 g = 1000 mg
ε	Empirical extinction coefficient of carotenoids, 250 $L/g \cdot cm^{-1}$
d	Cuvette optical path length, 1 cm
F	Dilution factor
W	Sample mass, g

2. Fresh Plant Leaves or Other Green Tissues Containing Chloroplasts

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

Carotenoid concentration: Cc (mg/L) = 4.367 × A470 - 0.014 × Ca - 0.454 × Cb

Carotenoid content (mg/g, mass) = Cc × Vextraction × F ÷ W

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

Symbol	Meaning
Vextraction	Volume of extract, 0.01 L
F	Dilution factor
W	Sample mass, g

Precautions

1. This 50T kit can test 48 samples.
2. Required instruments and supplies to be prepared by the user include a visible spectrophotometer, 1 mL glass cuvette, 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 has an effect, scan the sample extract at 400-700 nm using a spectrophotometer. If there is an absorption peak at 640-670 nm, chlorophyll is present; otherwise, it is absent.
4. When A exceeds 1, dilute the sample with extraction solution before measurement. Multiply the result by the dilution factor F in the calculation formula.
5. To avoid pigment decomposition caused by light exposure, protect the operation from light as much as possible. During grinding or homogenization, keep the processing time as short as possible.
6. The extraction solution is volatile. Take appropriate 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.