Plant Carotenoid Content Assay Kit

Note: Take two or three different samples for prediction before test.
Operation Equipment: Spectrophotometer/microplate reader
Catalog Number: BC4335
Size:100T/96S

Components:

Extract solution: 80% acetone, **self-prepared.** Mix with acetone: distilled water (V: V) = 4:1 for use.

Reagent I: Powder×1, storage at 4℃.

Product Description

Carotenoid is a kind of important natural pigment. It is widely found in the yellow, orange or red pigments of animals, higher plants, fungi and algae. Carotenoid is the precursor of vitamin A in vivo, and also has the functions of antioxidant, immune regulation, anticancer, reducing cardiovascular disease and colorant.

The carotenoids of plants exist in various yellow plastids or colored substances, such as yellow leaves, yellow flowers, yellow and red fruits and yellow tubers. Carotenoids in the sample are separated and extracted by solvent extraction. There is a special absorption peak at 440 ± 10 nm. Most of the chloroplasts of higher plants and algal microorganisms also contain carotenoids, which mainly absorb blue violet light, while chlorophyll A and chlorophyll B absorb both red and blue violet light. Therefore, in order to eliminate the interference of chlorophyll A and B on carotenoids, the content of chlorophyll A and chlorophyll B is calculated first according to the empirical formula, and then the content of carotenoids is further obtained; For tissues without chlorophyll, the carotenoid content can be calculated directly according to the empirical extinction coefficient of carotenoids.

Reagents and Equipment Required but Not Provided.

Spectrophotometer/microplate reader, desktop centrifuge, micro glass cuvette/96 well flat-bottom plate (non-polystyrene material), scales, adjustable pipette, mortar/homogenizer, 10 mL centrifuge tube/test tube, distilled water and acetone（>98%，AR）.

Procedure

I. Sample preparation

1. Wash the leaves (midvein removed) or other tissues of fresh plants with distilled water, then dry the surface water, weigh about 0.1 g, cut and put into a mortar/homogenizer.
2. Add 1 mL of distilled water, a small amount of Reagent I (about 10 mg), grind it under dark or weak light conditions, and transfer it into a 10 mL centrifuge tube or test tube.
3. Wash the mortar/homogenizer with the Extract solution, transfer all the flushing solution into the 10 mL centrifuge tube or test tube, use the Extract solution to make the volume up to 10mL. Put it

in the dark condition or cover it with tin foil paper for 3 hours (during which it can be mixed upside down for 2 times), observe that if the bottom tissue residue is close to white, the extraction is complete. If the tissue residue is not completely white, continue to extract until the color of the tissue residue is close to white.

II. Measurement steps

A、Determination of carotenoid content in yellow or other non-green tissues (excluding chloroplasts):

a.Preheat the spectrophotometer/microplate reader for 30 minutes, adjust the wavelength to 440 nm and adjust zero with **Extract solution.**

b.Sample test (blank tube is not needed for micro glass cuvette test, but only once for 96 well plate test).

Reagent name (μL)	Blank tube (B)	Test tube (T)
Sample	-	200
Extract solution	200	-
a. Detection with spectrophotometer: rapid determination of 440 nm for test tube in a micro glass cuvette, recorded as A_{440}. b. Detection with microplate reader: The absorbance value at 440 nm is measured rapidly in 96-well plate. It is recorded as A_{440B} and A_{440T} respectively, $\Delta A_{440} = A_{440T} - A_{440B}$.		

B. Determination of carotenoid content in fresh plant leaves or other green tissues (including chloroplasts):

a. Preheat the spectrophotometer/microplate reader for 30 minutes, adjust the multi wavelength to 470 nm, 646 nm and 663 nm, and adjust zero with extract for the spectrophotometer.

b. Sample test (blank tube is not needed for micro glass cuvette test, but only once for 96 well plate test).

Reagent name (μL)	Blank tube(B)	Test tube(T)
Sample	-	200
Extract solution	200	-
a. Detection with spectrophotometer: the absorbance values at 470 nm, 646 nm and 663 nm of test tube are determined rapidly in a micro glass cuvette, and are recorded as A_{470}, A_{646} and A_{663}, respectively. b. Detection with microplate reader: The absorbance values at 470 nm, 646 nm and 663 nm are measured rapidly in 96-well plate. They were recorded as A_{470B}, A_{470T}, A_{646B}, A_{646T}, A_{663B}, A_{663T}, $\Delta A_{470}=A_{470B}-A_{470B}$, $\Delta A_{646}= A_{646T}-A_{646B}$, $\Delta A_{663}= A_{663T}-A_{663B}$.		

Note: if there is residue in the upper extraction solution, take 1 mL of the upper extraction solution and put it in 1.5 mL Brown EP tube. Centrifugate it at 4000 r/min for 5 minutes at room temperature, and then take the supernatant for detection. If 96 well plate made of polystyrene is used, please complete the determination as soon as possible within 5 min after sample addition.

Calculation of Plant Carotenoid Content:

A. Formula for carotenoid content in yellow or other non-green tissues (excluding chloroplasts):

1. Calculated according to the micro glass cuvette:

Carotenoid content (mg/g fresh weight): $\Delta A_{440} \div (\varepsilon \times d) \times V_{ST} \times 1000 \div W \times F = 0.04 \times \Delta A_{440} \times F \div W$

V_{ST}: Total volume of extraction solution, 0.01 L;

1000: Unit conversion coefficient, 1 g=1000 mg;

ε: Empirical extinction coefficient of carotenoid, 250 L/g/cm;

d: Optical diameter of cuvette, 1 cm;

F: Dilution ratio;

W: Sample mass, g.

2. Calculated according to 96 well plate:

Carotenoid content (mg/g fresh weight): $\Delta A_{440} \div (\varepsilon \times d) \times V_{ST} \times 1000 \div W \times F = 0.067 \times \Delta A_{440} \times F \div W$

V_{ST}: Total volume of extraction solution, 0.01 L;

1000: Unit conversion coefficient, 1 g = 1000 mg;

ε: Carotenoid empirical extinction coefficient, 250 L/g/cm;

d: 96 well plate optical diameter, 0.6 cm;

F: Dilution ratio;

W: Sample mass, g.

B. Calculation formula of carotenoid content in leaves or other green tissues (including chloroplasts) of fresh plants:

1. Calculated according to the micro glass cuvette:

C_a (mg/L) $=12.21 \times \Delta A_{663} - 2.81 \times \Delta A_{646}$

C_b (mg/L) $=20.13 \times \Delta A_{646} - 5.03 \times \Delta A_{663}$

Carotenoid concentration: $C_c(mg/L) = (1000 \times \Delta A_{470} - 3.27 \times C_a - 104 \times C_b) \div 229$

$= 4.367 \times \Delta A_{470} - 0.014 \times C_a - 0.454 \times C_b$

Carotenoid content (mg/g fresh weight) $= C_c \times V_E \times F \div W = 0.01 \times C_c \times F \div W$

V_E: Volume of extraction solution, 0.01 L;

F: Dilution ratio;

W: Sample mass, g.

2. Calculated according to 96 well plate:

C_a (mg/L) $= (12.21 \times \Delta A_{663} - 2.81 \times \Delta A_{646}) \div 0.6 = 20.35 \times \Delta A_{663} - 4.83 \times \Delta A_{646}$

C_b (mg/L) $= (20.13 \times \Delta A_{646} - 5.03 \times \Delta A_{663}) \div 0.6 = 33.55 \times \Delta A_{646} - 8.38 \times \Delta A_{663}$

Carotenoid concentration: $C_c(mg/L) = (1000 \times \Delta A_{470} \div 0.6 - 3.27 \times C_a - 104 \times Cb) \div 229$

$= 7.278 \times \Delta A_{470} - 0.014 \times C_a - 0.454 \times Cb$

Carotenoid content (mg/g fresh weight) $= Cc \times V_E \times F \div W = 0.01 \times C_c \times F \div W$

V_E: Total volume of extraction solution, 0.01 L;

F: Dilution ratio;

W: Sample mass, g;

0.6: Light diameter ratio, 0.6 cm (96 well plate light diameter):1cm:(cuvette light diameter).

Note:

1. If it is uncertain whether there is chlorophyll influence in the tissue, the sample extract can be scanned with a spectrophotometer at the wavelength of 400-700 nm to see whether there is a wave peak between the wavelength of 640-670 nm, if there is a wave peak, there is chlorophyll, otherwise there is not.
2. If 96 well plate made of polystyrene is used, please complete the measurement as soon as possible within 5 minutes after sample adding.
3. When A is more than 1, it is recommended to dilute the sample with the extract and then conduct the determination, multiply the dilution factor F in the calculation formula.
4. In order to avoid light decomposition of pigment, avoid light as much as possible during operation, and shorten time as much as possible during grinding or homogenization.
5. the extract solution is volatile, and protective measures shall be taken during operation.

Experimental Examples:

1. Take 0.1 g of Daylily ， and add 1 mL of distilled water, a small amount of Reagent I (about 10 mg), grind it under dark or weak light conditions, and transfer it into a 10 mL centrifuge tube or test tube. Wash the mortar/homogenizer with the Extract solution, transfer all the flushing solution into the 10 mL centrifuge tube or test tube, use the Extract solution to make the volume up to 10mL. Put it in the dark condition or cover it with tin foil paper for 3 hours (during which it can be mixed upside down for 2 times),operate according to the determination steps A, measured by the 96 well flat-bottom plate A440=0.202,calculate the content::
   Plant Carotenoid Content（mg/g weight）= 0.067×A440÷W=0.1052 mg/g weight。
2. Take 0.1 g of Scindapsus ， and add 1 mL of distilled water, a small amount of Reagent I (about 10 mg), grind it under dark or weak light conditions, and transfer it into a 10 mL centrifuge tube or test tube. Wash the mortar/homogenizer with the Extract solution, transfer all the flushing solution into the 10 mL centrifuge tube or test tube, use the Extract solution to make the volume up to 10mL. Put it in the dark condition or cover it with tin foil paper for 3 hours (during which it can be mixed upside down for 2 times),operate according to the determination steps B, measured A470=0.415，A646=0.189，A663=0.379。
   Ca（mg/L）=20.35×0.379-4.83×0.189=6.1825 mg/L；
   Cb（mg/L）=33.55×0.189-8.38×0.379=2.0868 mg/L；
   Cc（mg/L）=7.278×ΔA470-0.014×Ca-0.454×Cb=1.6698 mg/L
   Plant Carotenoid Content（mg/g weight）= Cc×V 提取÷W =0.01×Cc÷W=0.167 mg/g weight

Related Products:

BC0990/BC0995	Plant Chlorophyll Content Assay Kit
BC2210/BC2215	Glyceraldehyde-3-phosphate Dehydrogenase(GAPDH) Activity Assay Kit

BC3400/BC3405 Pyrophosphate: Fructose-6-Phosphate-1-Phosphoric Acid Transferase(PFP) Activity Assay Kit