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Plant Carotenoid Content Assay Kit

Note: Take two or three different samples for prediction before test.
Operation Equipment: Spectrophotometer
Catalog Number: AK0080-50T-48S

Components:
Extract solution I: 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, desktop centrifuge, 1 mL glass cuvette, 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 for 30 minutes, adjust the wavelength to 440 nm and adjust zero with Extract.

b. Take 1 mL of the upper extraction solution and put it into 1 mL glass cuvette, measure the absorption value at 440 nm, and record it as A440.

B、 Determination of carotenoids in leaves or other green tissues (including chloroplasts) of fresh plants:

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

b. Take 1 mL of the upper extraction solution and put it into 1 mL glass cuvette, measure the absorption value at 470 nm, 646 nm and 663, and record it as A_{470}、 A_{646} and A_{663}.

Note: If there is residue in the upper extraction solution, take 1.2 mL of the upper extraction solution and put it in 1.5 mL brown EP tube. Centrifuge it at 4000 r/min for 5 minutes at room temperature, and then take the supernatant for detection.

III. 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): $A_{440}\div(\varepsilon\times d)\times V_{ST}\times 1000\div W\times F=0.04\times A_{440}\times F\div W$

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.

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.

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. 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.

3. 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.

4. the extract is volatile, and protective measures shall be taken during operation.

5. When measuring a large number of samples, pay attention to the liquid level position of the extract in the cuvette used for zero adjustment and correction to prevent errors caused by volatilization.

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 A440=0.316,calculate the content::

Plant Carotenoid Content（mg/g weight）= $0.04 \times A_{440} \div W$=0.1264 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=0.746, A646=0.285， A663=0.686 calculate the content::

Ca（mg/L）=12.21×0.686-2.81×0.285=7.5752 mg/L；

Cb（mg/L）=20.13×0.285-5.03×0.686=2.2865 mg/L；

Cc（mg/L）=4.367×0.746-0.014×Ca-0.454×Cb=2.1137 mg/L

Plant Carotenoid Content（mg/g weight）= Cc×V Extract÷W =0.01×Cc÷W=0.2114 mg/g weight