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Plant Carotenoid Content Assay Kit

Note: It is necessary to predict 2-3 large difference samples before the formal test.
Operation Equipment: Spectrophotometer
Catalog Number: AK0080-50T-48S

Product Composition: Before use, please carefully check whether the volume of the reagent is consistent with the volume in the bottle.

Reagent	Size	Preservation Condition
Extract solution	Liquid 85 mL×1	2-8℃
Reagent I	Powder×1	2-8℃

Product Description

Carotenoid is a general term for a class of important natural pigments. 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; mortar/homogenizer, desktop centrifuge, 1 mL glass cuvette, balance, adjustable pipette, EP tube/test tube, aluminum foil, distilled water.

Operation procedure:

I. Sample preparation(The sample size to be tested can be adjusted appropriately, and the specific proportion can be referred to the literature.)

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 the mortar/homogenizer.
2. According to the ratio of tissue mass (g) to extraction liquid volume (mL) of 1:5 to 15 (it is

recommended to weigh approximately 0.1g of tissue and add 1.5mL of extraction liquid), a small amount of reagent I (about 10mg) should be thoroughly ground under dark or weak light conditions and transferred into a 2mL EP tube.

3. Place it in the dark or wrap it with tin foil and extract at room temperature for 30 minutes. Observe that the bottom tissue residue is close to white, indicating complete extraction (during this period, it can be inverted and mixed several times to promote complete extraction). If the tissue residue has not completely turned white, continue extracting until the color of the tissue residue is close to white.

Note:

1. If there is residue in the upper extraction solution, it can be centrifuged at 4000r/min for 5 minutes at room temperature, and then take the supernatant for testing.

2. After completion of extraction, please test immediately. The evaporation of the extract solution and the degradation of chlorophyll will affect the results. Testing as soon as possible can reduce interference.

3. The extract is volatile. If the volume decreases after grinding, it can be replenished with the extract to a final volume of 1.5 mL.

II. Determination procedure:

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

1.Preheat the spectrophotometer for more than 30 minutes, adjust the wavelength to 440 nm and set zero with extract solution.

2.Take 1 mL of the upper extract solution and put it into 1 mL glass cuvette, measure the absorption value at 440 nm, and record it as A_{440}.

B. Carotenoids in leaves of fresh plants or other green tissues (including chloroplasts):

1. Preheat the spectrophotometer for more than 30 minutes, adjust the wavelength to 470 nm, 646 nm and 663 nm, and set zero with extract solution.

2. 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}.

III. Calculate the content of Carotenoid:

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

Carotenoid content (mg/g mass)=$\Delta A_{440}\div(\varepsilon\times d)\times V_{ST}\times 1000\div W\times F=0.006\times\Delta A_{440}\times F\div W$

V_{ST}: Total volume of extract solution, 0.0015L; 1000: Unit conversion coefficient, 1 g=1000 mg; ε: Empirical extinction coefficient of carotenoid, 250 L/g/cm; d: Glass cuvette optical diameter, 1 cm; F: Dilution multiple; W: Sample mass, g.

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

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 mass) = $C_c\times V_E\times F\div W= 0.0015\times C_c\times F\div W$

V_E: Volume of extract solution, 0.0015L; F: Dilution multiple; W: Sample mass, g.

Note:

1. If it is uncertain whether there is chlorophyll influence in the tissue, the sample extract solution 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 a mortar is used for extraction, it is recommended to rinse the mortar with the extraction solution until all the green substances are transferred to the EP tube. Record the final volume of the extraction solution used and note to modify the calculation formula accordingly.
3. If the absorbance value exceeds 1.2 during the measurement, the extraction solution can be diluted appropriately; when the absorbance value is less than 0.05, the amount of V extraction can be reduced appropriately, or the sample tissue quantity can be increased. Please note to simultaneously modify the calculation formula.
4. 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:

Sample	Mass（g）	Dilution multiple	A_{440}	Carotenoid Content	Unit
Carrot	0.1006	2	0.791	0.094	mg/g mass
Corn kernel	0.1045	1	0.508	0.029	mg/g mass

Note:

1. Pre-treatment process of carrots and corn kernels: Add 1.5 mL of the extraction solution and thoroughly grind in the dark. Perform 30-minute extraction at room temperature in the dark, with 2-3 times of shaking and mixing during the process. After the extraction is completed, centrifuge at 4000 rpm for 5 minutes, and take the supernatant for detection.
2. Use the 1 mL glass cuvette to measure.

Recent Protect Citations:

[1] Song K, Zhou Z, Huang Y, Chen L, Cong W. Multi-omics insights into the mechanism of the high-temperature tolerance in a thermotolerant Chlorella sorokiniana. Bioresour Technol. 2023 Dec;390:129859. doi: 10.1016/j.biortech.2023.129859. Epub 2023 Oct 12. PMID: 37832851.