Plant Flavonoids Colorimetric Assay Kit

Catalog No: E-BC-K284-S

Method: Colorimetric method

Specification: 100 Assays (Can detect 86 samples without duplication)

Instrument: Spectrophotometer

Sensitivity: 0.315 μg/mL

Detection range: 0.315-150 μg/mL

- Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

General information

▲Intended use

This kit can be used to measure the Flavonoids content in plant tissue samples.

▲Background

Flavonoids are common plant secondary metabolites, such as red, blue, and purple anthocyanins in plant tissues. Flavonoids can scavenge free radicals directly by hydrogen atoms. The ability of oxidation resistance of many flavonoids is higher than vitamin C and vitamin E.

▲Detection principle

In alkaline nitrite solution, flavonoids form red complex with aluminum ion. The flavonoid content of the sample can be calculated by measuring the absorptivity of the sample extract at 510 nm.

Kit components & storage

Item	Component	Specification	Storage
Reagent 1	1 mg/mL Standard	2 mL × 1 vial	2-8℃ , 6 months
Reagent 2	Saline Solution	1.8 mL × 2 vials	2-8℃ , 6 months
Reagent 3	Aluminium Reagent	1.8 mL × 2 vials	2-8℃ , 6 months
Reagent 4	Alkali Reagent	50 mL × 1 vial	2-8℃ , 6 months

Note: The reagents must be stored strictly according to the preservation conditions in the above table. The reagents in different kits cannot be mixed with each other.

Materials prepared by users

Instruments

Spectrophotometer (510 nm), Vortex mixer, Micropipettor, Air oven , Crusher, Ultrasonic cell processor

Consumptive material

Tips (10 μL, 200 μL, 1000 μL), EP tubes (1.5 mL, 2 mL, 5 mL)

Reagents

Double distilled water, 60% Ethanol

Safety data

Some of the reagents in the kit contain dangerous substances. It should be avoided to touch the skin and clothing. Wash immediately with plenty of water if touching it carelessly. All the samples and waste material should be treated according to the relevant rules of laboratory's biosafety.

Precautions

Before the experiment, please read the instructions carefully, and wear gloves and work clothes.

Pre-assay preparation

Sample preparation

1. Drying and crushing of plant tissues

Weigh 5-10 g fresh plant tissue and wash with distilled water, absorb moisture on the surface of tissue with filter paper, then put in a vacuum dryer and dry to constant weight at 80℃ . Crush the sample and filter over 40 mesh screen, sealed at room temperature.

2. Extraction of plant tissue

Accurately weigh 0.02 g sample in step 1, add 2 mL of 60% alcohol (self-prepared), then shake at 60℃ for 2 hours with constant temperature shaking incubator. Centrifuge at 10000 g for 10 min (25℃), then take the supernatant for detection. Or treat the sample with ultrasonic cell disruptor (power: 300W, 3 seconds/time, interval for 4 seconds, repeat for 30 min), then centrifuge at 10000 g for 10 min (25℃), then take the supernatant for detection.

▲Dilution of sample

It is recommended to take 2~3 samples with expected large difference to do pre-experiment before formal experiment and dilute the sample according to the result of the pre-experiment and the detection range (0.315-150 μg/mL).

The recommended dilution factor for different samples is as follows (for reference only)

Sample type	Dilution factor
Camphor leaves tissue homogenization	8-15
Carrot tissue homogenization	2-5
Green pepper tissue homogenization	1

Note: The diluent is normal 60% Ethanol .

Assay protocol	
Ambient temperature	25-30℃
Optimum detection wavelength	510 nm

Instructions for the use of transferpettor:

(1) Equilibrate the pipette tip in that reagent before pipetting each reagent.

(2) Don’t add the liquid outside the tips into the reaction system when pipetting each reagent.

Assay protocol

▲Operating steps

The preparation of standard curve

Dilute 1 mg/mL Standard solution with double distilled water to a serial concentration. The recommended dilution gradient is as follows: 20, 60, 80, 100, 120, 150 μg/mL.

The measurement of samples

(1) Standard tube: Add 0.54 mL of standard solution with different concentrations into the 2 mL EP tubes.
Sample tube: Add 0.54 mL of Sample into a 2 mL EP tube.
Blank tube: Add 0.54 mL of double distilled water into a 2 mL EP tube.

(2) Add 0.03 mL of reagent 2 into each tube, oscillate fully and stand for 5 min at room temperature.

(3) Add 0.03 mL of reagent 3 into each tube, oscillate fully and stand for 5 min at room temperature.

(4) Add 0.4 mL of reagent 4 into each tube, oscillate fully and stand for 15 min at room temperature.

(5) Set the spectrophotometer to zero with double distilled water and measure the OD values of each tube at 510 nm with 0.5 cm optical path cuvette.

Operation table

	Standard tube	Sample tube	Blank tube
Double distilled water (mL)			0.54
Sample (mL)		0.54	
Standard with different concentrations (mL)	0.54		
Reagent 2 (mL)	0.03	0.03	0.03
Oscillate fully and stand for 5 min at room temperature.			
Reagent 3 (mL)	0.03	0.03	0.03
Oscillate fully and stand for 5 min at room temperature.			
Reagent 4 (mL)	0.4	0.4	0.4
Oscillate fully and stand for 15 min at room temperature. Set the spectrophotometer to zero with double distilled water and measure the OD values of each tube at 510 nm with 0.5 cm optical path cuvette.			

▲Calculation

Plot the standard curve by using OD value of standard and correspondent concentration as y-axis and x-axis respectively. Create the standard curve with graph software (or EXCEL). The concentration of the sample can be calculated according to the formula based on the OD value of sample.

The standard curve is: $y = ax + b$.

$$\text{Flavonoids content (mg/g tissue)} = (\Delta A_{510} - b) \div a \times V \div W \div 1000 \times f$$

Note:

y: The absolute OD value of standard ($OD_{Standard} - OD_{Blank}$)

x: The concentration of standard

a: The slope of standard curve

b: The intercept of standard curve

ΔA_{510}: $OD_{Sample} - OD_{Blank}$

V: the volume of 60% alcohol in the pretreatment of sample, 2 mL

W: weight of sample, 0.02 g

1000: unit conversion (μg → mg)

f: the dilution multiple of tested samples

▲Notes

1. This kit is for research use only.
2. Instructions should be followed strictly, changes of operation may result in unreliable results.
3. The validity of kit is 6 months.
4. Do not use components from different batches of kit.

Appendix I Performance characteristics

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Detection range	0.315-150 μg/mL	Average intra-assay CV (%)	1.9
Sensitivity	0.315 μg/mL	Average inter-assay CV (%)	2.2
Average recovery rate (%)	98		

▲Example analysis

For daucus carota tissue, take the sample pretreated and dilute the sample with 60% ethanol for 2 times, take 0.54 mL of diluted sample, carry the assay according to the operation table.

The results are as follows:

standard curve: y = 0.0029 x + 0.0008 (R^2=0.9993), the average OD value of the sample well is 0.203, the average OD value of the blank well is 0.0025, the calculation result is:

Flavonoids content (mg/g tissue)
= (0.203-0.0025-0.0008)÷0.0029×2÷0.02÷1000×2=13.77 mg/g tissue