Plant Flavonoids Colorimetric Assay Kit

Catalog No: E-BC-K284-M

Method: Colorimetric method

Specification: 96T (Can detect 80 samples without duplication)

Measuring instrument: Microplate reader

Sensitivity: 0.66 μg/mL

Detection range: 0.66-150 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 absorbance of the sample extract at 510 nm.

Kit components & Storage

Item	Component	Specification	Storage
Reagent 1	1 mg/mL Standard	1.8 mL × 1 vial	2-8℃ , 6 months
Reagent 2	Saline Solution	1 mL × 2 vials	2-8℃ , 6 months
Reagent 3	Aluminium Reagent	1.8 mL × 2 vials	2-8℃ , 6 months
Reagent 4	Alkali Reagent	30 mL × 1 vial	2-8℃ , 6 months
	Microplate	96 wells	No requirement
	Plate Sealer	2 pieces	

Note: The reagents must be stored strictly according to the preservation conditions in the above table. Extraction solution (60% alcohol) in not provided in this kit.

Materials prepared by users

Instruments

Microplate reader (500-520 nm), Micropipettor, Vortex mixer, Centrifuge, Vacuum dryer, Ultrasonic cell disruptor.

Consumptive material

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

Reagents

Double distilled water, 60% alcohol, absolute 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

▲The key point of the assay

1. After adding reagent 2 or 3, it must be stood at room temperature for 5 minutes before adding other reagents.

2. When adding reagent 4, it was allowed to stand at room temperature for 15 min.

Pre-assay preparation

▲Reagent preparation

Bring all the reagents to room temperature before use.

▲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 1500 g for 10 min, 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, 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 (0.66-150 μg/mL).

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

Sample type	Dilution factor
Epipremnum aureum	10-15
Green pepper	1
Pumpkin	1
Heather	25-35

Note: The diluent is 60% alcohol.

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

▲Plate set up

	1	2	3	4	5	6	7	8	9	10	11	12
A	A	A	S1	S9	S17	S25	S33	S41	S49	S57	S65	S73
B	B	B	S2	S10	S18	S26	S34	S42	S50	S58	S66	S74
C	C	C	S3	S11	S19	S27	S35	S43	S51	S59	S67	S75
D	D	D	S4	S12	S20	S28	S36	S44	S52	S60	S68	S76
E	E	E	S5	S13	S21	S29	S37	S45	S53	S61	S69	S77
F	F	F	S6	S14	S22	S30	S38	S46	S54	S62	S70	S78
G	G	G	S7	S15	S23	S31	S39	S47	S55	S63	S71	S79
H	H	H	S8	S16	S24	S32	S40	S48	S56	S64	S72	S80

[Note]: A-H, standard wells; S1-S80, sample wells.

▲Operating steps

The preparation of standard curve

Dilute 1 mg/mL standard solution with absolute ethanol to a serial concentration. The recommended dilution gradient is as follows: 0, 20, 40, 60, 80, 100, 120, 150 μg/mL.

The measurement of samples

1. Standard well: Add 75 μL of standard solution with different concentrations.
   Sample well: Add 75 μL of Sample.

2. Add 10 μL of reagent 2 into each well, oscillate fully and stand for 5 min at room temperature.

3. Add 30 μL of reagent 3 into each well, oscillate fully and stand for 5 min at room temperature.

4. Add 180 μL of reagent 4 into each well, oscillate fully and stand for 15 min at room temperature.

5. Measure the OD value of each well at 510 nm with microplate reader.

▲Operation table

	Standard well	Sample well
Standard with different concentrations (μL)	75	
Sample (μL)		75
Reagent 2 (μL)	10	10
Oscillate fully and stand for 5 min at room temperature.		
Reagent 3 (μL)	30	30
Oscillate fully and stand for 5 min at room temperature.		
Reagent 4 (μL)	180	180
Oscillate fully and stand for 15 min at room temperature. Measure the OD values of each well at 510 nm with microplate reader.		

▲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.

Flavonoids content (mg/g tissue)= ($\triangle A_{510}$-b)÷a×V÷W÷1000×f

Note:

y: $OD_{standard}$-OD_{blank}. (OD_{Blank} is the OD value when the standard concentration is 0)

x: the concentration of standard.

a: the slope of the standard curve.

b: the intercept of standard curve.

$\triangle A_{510}$: 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	0.66-150 μg/mL	Average intra-assay CV (%)	4.0
Sensitivity	0.66 μg/mL	Average inter-assay CV (%)	5.3
Average recovery rate (%)	103		

▲Example analysis

The supernatant of epipremnum aureum tissue was diluted with 60% anhydrous ethanol for 10 times. Take 75 μL of diluted sample, and carry the assay according to the operation table. The result is as follows: Standard curve: y= 0.0025 x - 0.0052. The average OD value of the blank well is 0.04, the average value of the sample well is 0.186, and the calculation result is:

Flavonoids content (mg/g tissue)=(0.816-0.04+0.0052)÷0.0025×2÷0.02÷1000×10

=60.48mg/g tissue