Technical Manual

Plant Flavonoids Colorimetric Assay Kit

- Catalogue Code: MAES0171
- Size: 96T
- Research Use Only

1. Key features and Sample Types

Detection method:

Colorimetric method

Specification:

96T

Range:

0.66-150 µg/mL

Sensitivity:

0.66 µg/mL

Storage:

2-8°C for 6 months

Expiry:

See Kit Label

Experiment Notes:

This kit is for **research use only.**

Instructions should be strictly followed. Changes of operation may result in unreliable results.

The validity of kit is 6 months.

Do not use components from different batches of kit.

2. 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.

3. Intended Use

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

4. 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.

5. Kit components & storage

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

Materials required but not supplied

- Micropipettor
- Incubator
- Centrifuge
- Microplate Reader (500-520 nm)
- Tips (10 µL, 200 µL, 1000 µL)
- EP tubes (1.5 mL, 2 mL)
- Double distilled water
- 60% Alcohol,
- Absolute ethanol

6. Assay Notes:

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

2. When adding alkali reagent, allow to stand at room temperature for 15 min.

7. Reagent preparation:

Bring all reagents to room temperature before use.

8. 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°C. 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°C 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.

Sample Notes:

The concentration should be determined before preforming the assay. If the sample concentration is not within the range of the standard curve, users must determine the optimal sample dilutions for their particular experiments.

If the sample type is not included in the manual, a preliminary experiment is suggested to verify the validity.

Dilution of Samples:

Large variances in results may be seen when performing pre-experiments. 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;

9. Assay Protocol

Ambient Temperature: 25-30°C

Optimum detection wavelength: 510 nm

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.

10. Operation Steps

The preparation of standard curve

Dilute standard solution (1 mg/mL) 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 standards with different concentrations to the corresponding wells.
   Sample well: add 75 μL of sample to the corresponding wells.

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

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

4. Add 180 μL of alkali reagent 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
Saline solution (μL)	10	10
Oscillate fully and stand for 5 min at room temperature.		
Aluminium reagent (μL)	30	30
Oscillate fully and stand for 5 min at room temperature.		
Alkali reagent (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.		

11. Calculations

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)

$$= (\triangle A_{510}\text{-}b) \div a \times V \div W \div 1000 \times f$$

x: the concentration of standard

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

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

12. Performance Characteristics

Detection Range	0.66-150 μg/mL
Sensitivity	0.66 μg/mL
Average recovery rate (%)	103
Average inter-assay CV (%)	5.3
Average intra-assay CV (%)	4.0

Analysis

The supernatant of epipremnum aureum tissue was diluted with 60% absolute ethanol for 10 times. Take 75 μL of diluted sample, carry the assay according to the operation table.

The results are 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)

= (0.186 - 0.04 + 0.0052) ÷ 0.0025 × 2 ÷ 0.02 ÷ 1000 × 10 = 60.48 mg/g

Safety Notes

Some of the reagents in the kit contain dangerous substances. Prevent touching 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.

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

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