TECHNICAL MANUAL

Plant Flavonoids Colorimetric Assay Kit

- **SKU CODE:** MAES0171
- **SIZE:** 48 Tests
- **DETECTION PRINCIPLE:** Assay Kit
- **RUO:** Research-Use-Only

Revised: 01/2026

1. Assay summary

- ![img-0.jpeg](img-0.jpeg)

2. Intended use

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

3. Detection principle

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

4. Kit components & storage

Item	Component	Size 1 (48 T)	Size 2 (96 T)	Size 3 (500Assays)	Storage
Reagent 1	1 mg/mL Standard	1.8 mL×1 vial	1.8 mL×1 vial	9 mL×1 vial	2-8°C, 12 months
Reagent 2	Saline Solution	1 mL×1 vial	1 mL × 2 vials	10 mL×1 vial	2-8°C, 12 months
Reagent 3	Aluminum Reagent	1.8 mL×1 vial	1.8 mL×2 vials	18 mL×1 vial	2-8°C, 12 months
Reagent 4	Alkali Reagent	15 mL×1 vial	30 mL×1 vial	50 mL×3 vials	2-8°C, 12 months
	Microplate	48 wells	96 wells	/	No requirement
	Plate Sealer	2 pieces	2 pieces	2 pieces	

5. Materials prepared by users

Instruments:

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

Reagents:

Double distilled water, 60% alcohol, absolute ethanol

6. Reagent preparation

1. Equilibrate all reagents to room temperature before use.
2. **Preparation of the standard curve:** Always prepare a fresh set of standards. Discard working standard dilutions after use. 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.

7. Sample preparation

1. **Sample preparation:** 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 place in a vacuum dryer and dry to constant weight at 80°C. Crush the sample and filter through a 40 mesh screen, then store sealed at room temperature. Extraction of Plant tissue: Accurately weigh 0.02 g sample from step 1, add 2 mL of 60% alcohol (self-prepared), then shake at 60°C for 2 hours with a constant temperature shaking incubator. Centrifuge at 1500×g for 10 min, then take the supernatant for detection. Alternatively, treat the sample with an ultrasonic cell disruptor (power: 300 W, 3 seconds/time, interval for 4 seconds, repeat for 30 min), then centrifuge at 10000×g for 10 min, and take the supernatant for detection.
2. **Dilution of sample:** 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. For the dilution of other sample types, please perform a pretest to confirm the dilution factor.

8. The key points of the assay

1. After adding saline solution or aluminum reagent, allow to stand at room temperature for 5 minutes before adding other reagents.
2. When adding alkali reagent, allow to stand at room temperature for 15 min.

9. Operating steps

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

2. Add 10 µL of saline solution into each well, mix thoroughly and stand for 5 min at room temperature.
3. Add 30 µL of aluminum reagent into each well, mix thoroughly and stand for 5 min at room temperature.
4. Add 180 µL of alkali reagent into each well, mix thoroughly and stand for 15 min at room temperature.
5. Measure the OD value of each well at 510 nm with a microplate reader.

10. Calculation

The standard curve:

1. Average the duplicate reading for each standard.

2. Subtract the mean OD value of the blank (Standard # ①) from all standard readings. This is the absolute OD value.

3. Plot the standard curve by using the absolute OD value of standard and corresponding concentration as y-axis and x-axis respectively. Create the standard curve (y = ax + b) with graph software (or EXCEL).

The sample:

Flavonoids content (mg/g tissue) = (ΔA_510 - b) ÷ a × V ÷ W ÷ 1000 × f

[Note]

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.

11. Appendix I Performance Characteristics

Parameter:

Intra-assay Precision

Three human serum samples were assayed in replicates of 20 to determine precision within an assay. (CV = Coefficient of Variation)

Inter-assay Precision

Three human serum samples were assayed 17 times in duplicate by three operators to determine precision between assays.

Recovery

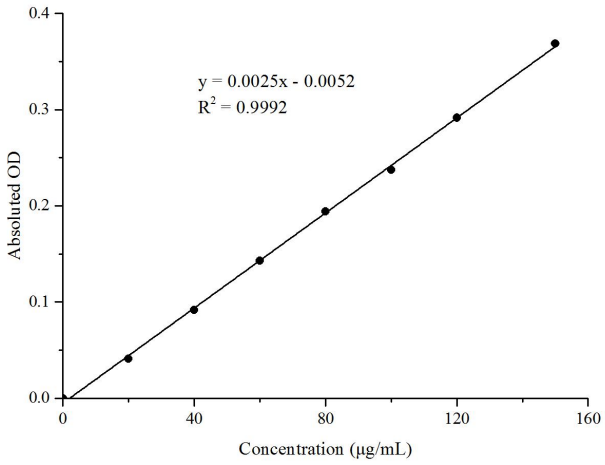
Three samples of high concentration, middle concentration and low concentration were tested with each concentration performed 6 times in parallel to obtain an average recovery rate of 103%.

Sensitivity

The analytical sensitivity of the assay is 0.66 µg/mL. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 20 times, and calculating the corresponding concentration.

Standard curve data

As the OD value of the standard curve may vary according to the conditions of the actual assay performance (e.g. operator, pipetting technique or temperature effects), the standard curve and data are provided below for reference only:



12. Appendix II Example Analysis

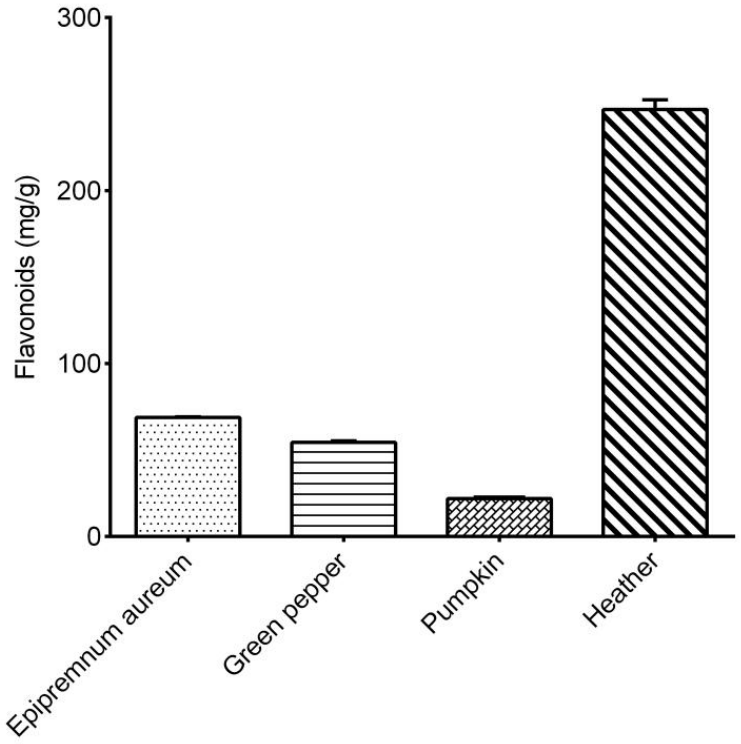
Example analysis:

The supernatant of epipremnum aureum tissue was diluted with 60% absolute ethanol by 10 times. Take 75 µL of diluted sample, and perform the assay according to the operation steps. The results are as follows:

Standard curve: y = 0.0025x - 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

Detect the supernatant of epipremnum aureum (diluted 10 times), supernatant of green pepper tissue, supernatant of pumpkin tissue and supernatant of heather tissue (diluted 30 times) according to the protocol, the result is as follows:



13. Statement

1. This assay kit is for Research Use Only. Assay Genie assumes no responsibility for any problems or legal liabilities arising from the use of this kit for clinical diagnosis or any other purpose.
2. Please read the instructions carefully and calibrate the instruments before performing the experiments. Follow the instructions strictly throughout the procedure.
3. Appropriate protective measures must be taken, including wearing a lab coat and latex gloves.
4. If the concentration of the substance falls outside the detection range, perform an additional dilution or concentration step on the sample.

5. It is recommended to perform a pre-test if your sample type is not listed in the instruction manual.
6. Experimental results are closely related to reagent quality, operator technique, environmental conditions, and other factors. Assay Genie guarantees the quality of the kits only and is NOT responsible for sample consumption resulting from use of the assay kits. It is advisable to estimate the expected sample usage and reserve sufficient samples before starting the experiment.

Assay Genie 100% money-back guarantee!

If you are not satisfied with the quality of our products and our technical team cannot resolve your problem, we will give you 100% of your money back.

Manufacturers Statement: This final kit system is assembled and quality-released by Assay Genie Limited.