Plant Flavonoids Colorimetric Assay Kit

Catalog Number EEA057(100 assays)

Rev 1.0

For safety and biohazard guidelines, read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Product description:

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

Contents and storage

Kit and components are shipped at 2-8 ℃. An unopened kit can be stored at 2-8 ℃ for 12 months.

Components	Quantity (100 assays)
1 mg/mL Standard	2 mL
Saline Solution	4 mL
Aluminium Reagent	4 mL
Alkali Reagent	50 mL

Required materials

- Distilled or deionized water
- 60% Ethanol
- Spectrophotometer with measurement capability at 510 nm
- Vortex mixer, Micropipettor, Centrifuge, Water bath
- Calibrated adjustable precision pipettes and glass or plastic tubes for diluting solution

.

Procedural guidelines

IMPORTANT! Reagents are lot-specific. Do not mix or interchange different reagent lots from various kit lots.

Allow reagents to reach room temperature before use. Mix to redissolve any precipitated salts.

Sample preparation guidelines

Drying and crushing of plant tissues:

Weigh 5-10 g fresh plant tissue and wash with distilled or deionized 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.

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: 300 W, 3 s/time, interval for 4 s, repeat for 30 min), then centrifuge at 10000×g for 10 min, then take the supernatant for detection.

Prepare samples

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

Note: Use all samples within 2 hours of dilution.

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

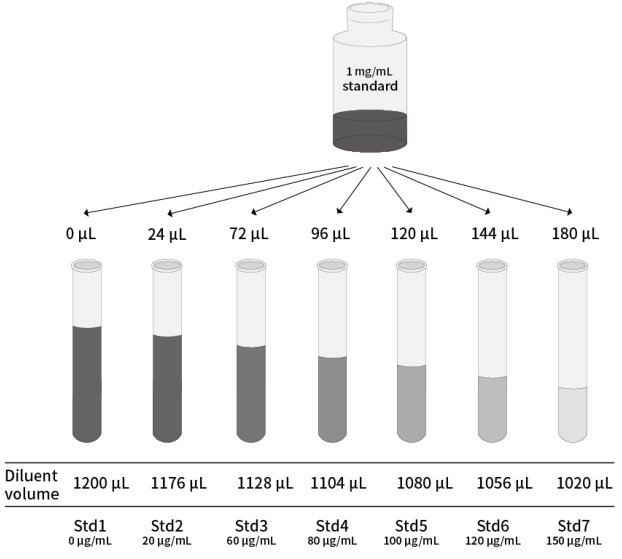
Sample type	Dilution factor
Camphor leaves tissue homogenization	8-15
Carrot tissue homogenization	2-5
Green pepper tissue homogenization	1

Note: The diluent is 60% ethanol.

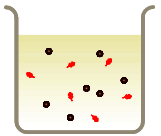
Prepare diluted standards

Note: Use glass or plastic tubes for diluting standards.

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



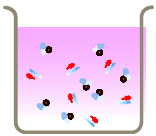
Assay procedure



1. **Add standard and sample**

a. Standard tube: Add 0.54 mL of standard solution with different concentrations into the 2 mL EP tubes.

b. Sample tube: Add 0.54 mL of sample into a 2 mL EP tube.



2. Add substrate

a. Add 0.03 mL of saline solution into each tube, oscillate fully and stand for 5 min at room temperature.

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

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

d. Set the spectrophotometer to zero with distilled or deionized water and measure the OD values of each tube at 510 nm with 0.5 cm optical path cuvette.

Calculation

1. Average the duplicate reading for each standard.
2. Subtract the mean OD value of the blank (Std1) from all standard readings. This is the absoluted OD value.
3. Plot the standard curve by using absoluted OD value of standard and correspondent concentration as y-axis and x-axis respectively. Create the standard curve (y = ax + b) with graph software (or EXCEL).

Tissue sample:

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

[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 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

To easy calculate the test results, refer to the calculation file available on the webpage.

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, and carry the assay according to the operation steps. The results are as follows:

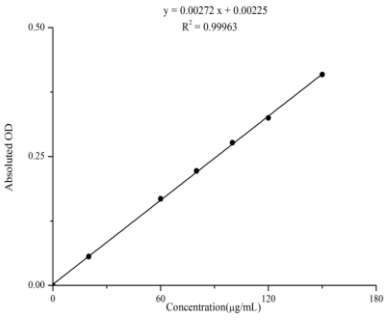
standard curve: y = 0.0029 x + 0.0008 (R^2=0.9993), the average OD value of the sample well is 0.203, the average OD value of the blank well is 0.0025, and the calculation result is:

$$\text{Flavonoids content (mg/g tissue)} =$$
$$(0.203 - 0.0025 - 0.0008) \div 0.0029 \times 2 \div 0.02 \div 1000 \times 2$$
$$= 13.77 \text{ mg/g tissue}$$

Performance characteristics

▪Standard curve (example)

The following data were obtained for the various standards over the range of 0–150 μg/mL standard.

Concentration (μg/mL)	ΔA at 510 nm	Standard Curve
150	0.410	
120	0.326	
100	0.278	
80	0.223	
60	0.169	
20	0.057	
0	0.001	

▪Intra-assay Precision

Three green pepper samples were assayed in replicates of 20 to determine precision within an assay.

Parameters	Sample 1	Sample 2	Sample 3
Mean(μg/mL)	5.80	75.60	126.90
%CV	2.3	1.8	1.6

CV = Coefficient of Variation

▪Inter-assay Precision

Three green pepper samples were assayed 20 times in duplicate by three operators to determine precision between assays.

Parameters	Sample 1	Sample 2	Sample 3
Mean(μg/mL)	5.80	75.60	126.90
%CV	1.8	2.3	2.5

CV = Coefficient of Variation

▪Expected values

This assay was tested with green pepper samples without dilutions.

Sample Type	Range (**mg/g tissue**)	Average (**mg/g tissue**)
Green pepper tissue homogenization	8.3-12.3	10.1

▪Recovery

Take three samples of high concentration, middle concentration and low concentration to test the samples of each concentration for 6 times parallelly to get the average recovery rate of 98%.

	Sample 1 (low conc.)	Sample 2 (middle conc.)	Sample 3 (high conc.)
Expected Conc. (μg/mL)	45.5	95.5	114
Observed Conc. (μg/mL)	46.0	91.7	110.6
Recovery rate (%)	101	96	97

▪Sensitivity

The analytical sensitivity of the assay is 0.315 µg/mL. This was determined by adding two standard deviations to the mean OD obtained when the zero standard was assayed 20 times and calculating the corresponding concentration.

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Corporate entity: Bender MedSystems GmbH | Campus Vienna Biocenter 2 | 1030 Vienna, Austria

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