（FOR RESEARCH USE ONLY. DO NOT USE IT IN CLINICAL DIAGNOSIS !）

Catalog No：E-BC-K284-M

Specification：48T(32 samples)/96T(80 samples)/ 500Assays(484 samples)

Measuring instrument：Microplate reader（500-520 nm）

Detection range：0.66-150 μg/mL

Elabscience® Plant Flavonoids Colorimetric Assay Kit

This manual must be read attentively and completely before using this product.

If you have any problem, please contact our Technical Service Center for help：

Toll-free: 1-888-852-8623
Tell: 1-832-243-6086
Fax: 1-832-243-6017
Email: techsupport@elabscience.com
Website: www.elabscience.com

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

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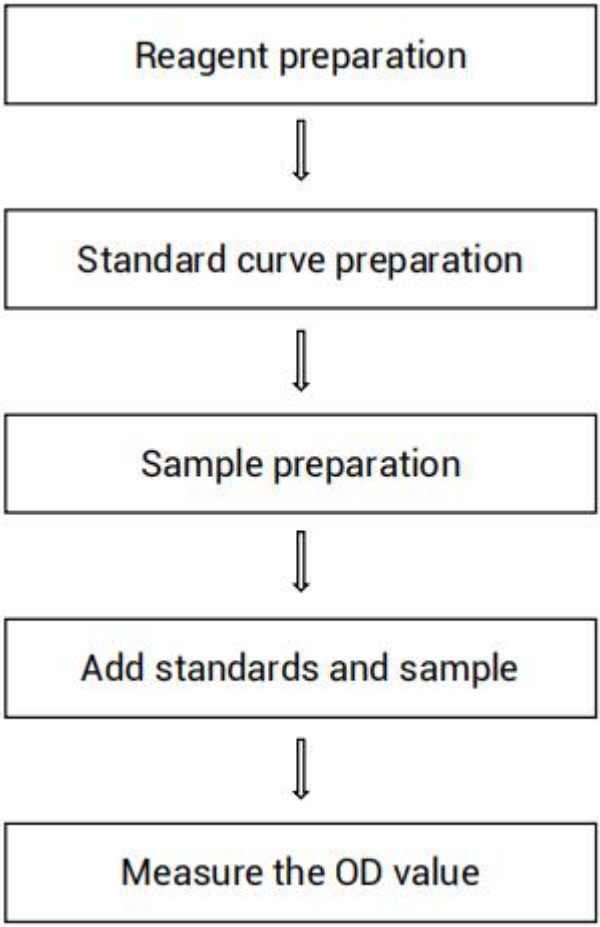
Assay summary



Intended use

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

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	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℃, 12 months
Reagent 2	Saline Solution	1 mL×1 vial	1 mL × 2 vials	10 mL×1 vial	2-8℃, 12 months
Reagent 3	Aluminium Reagent	1.8 mL×1 vial	1.8 mL×2 vials	18 mL×1 vial	2-8℃, 12 months
Reagent 4	Alkali Reagent	15 mL×1 vial	30 mL×1 vial	50 mL×3 vials	2-8℃, 12 months
	Microplate	48 wells	96 wells	/	No requirement
	Plate Sealer	2 pieces			
	Sample Layout Sheet	1 piece			

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. For a small volume of reagents, please centrifuge before use, so as not to obtain sufficient amount of reagents.

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

Reagent preparation

① Equilibrate all the reagents to room temperature before use.

② The preparation of 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. Reference is as follows:

Item	①	②	③	④	⑤	⑥	⑦	⑧
Concentration (μg/mL)	**0**	**20**	**40**	**60**	**80**	**100**	**120**	**150**
1 mg/mL potassium standard (μL)	0	24	48	72	96	120	144	180
Absolute ethanol (μL)	1200	1176	1152	1128	1104	1080	1056	1020

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 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 seconds/time, interval for 4 seconds, repeat for 30 min), then centrifuge at 10000×g for 10 min, then 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 do pretest to confirm the dilution factor

The key points of the assay

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, it was allowed to stand at room temperature for 15 min.

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, 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.

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 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).

The sample:

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

[Note]

$\triangle A_{510}$: 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.

Appendix I Performance Characteristics

1. Parameter:

Intra-assay Precision

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

Parameters	Sample 1	Sample 2	Sample 3
Mean (μg/mL)	2.60	34.50	85.40
%CV	4.5	4.2	3.3

Inter-assay Precision

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

Parameters	Sample 1	Sample 2	Sample 3
Mean (μg/mL)	2.60	34.50	85.40
%CV	5.6	4.8	5.5

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 103%.

	Standard 1	Standard 2	Standard 3
Expected Conc. (μg/mL)	26	75	116
Observed Conc. (μg/mL)	27.3	77.3	117.2
recovery rate(%)	105	103	101

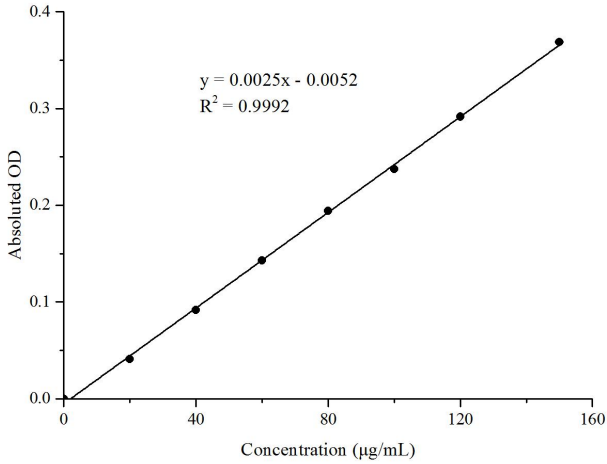
Sensitivity

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.

2. Standard curve:

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), so the standard curve and data are provided as below for reference only：

Concentration (μg/mL)	0	20	40	60	80	100	120	150
OD value	0.041	0.082	0.132	0.181	0.235	0.272	0.338	0.405
	0.040	0.083	0.134	0.183	0.233	0.281	0.340	0.413
Average OD	0.040	0.082	0.133	0.182	0.234	0.277	0.339	0.409
Absoluted OD	0.000	0.042	0.093	0.142	0.194	0.237	0.299	0.369



Appendix Π Example Analysis

Example analysis：

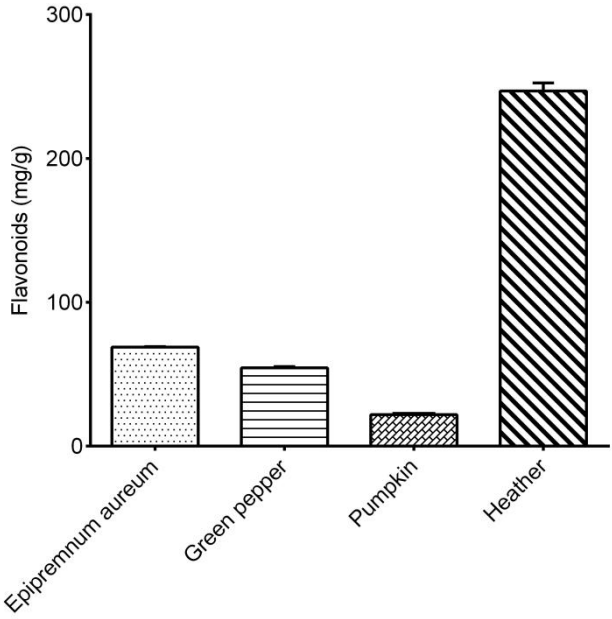
The supernatant of epipremnum aureum tissue was diluted with 60% absolute ethanol for 10 times. Take 75 μL of diluted sample, and carry the assay according to the operation steps. The results are as follows:

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

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



Statement

1. This assay kit is for Research Use Only. We will not response for any arising problems or legal responsibilities causing by using the kit for clinical diagnosis or other purpose.
2. Please read the instructions carefully and adjust the instruments before the experiments. Please follow the instructions strictly during the experiments.
3. Protection methods must be taken by wearing lab coat and latex gloves.
4. If the concentration of substance is not within the detection range exactly, an extra dilution or concentration should be taken for the sample.
5. It is recommended to take a pre-test if your sample is not listed in the instruction book.
6. The experimental results are closely related to the situation of reagents, operations, environment and so on. Elabscience will guarantee the quality of the kits only, and NOT be responsible for the sample consumption caused by using the assay kits. It is better to calculate the possible usage of sample and reserve sufficient samples before use.