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

Catalog No：E-BC-K354-M

Specification：96T(40 samples)/ 500Assays(242 samples)

Measuring instrument：Microplate reader（750-770 nm）

Detection range：1.05-148 μg/mL

Elabscience® Total Phenols Colorimetric Assay Kit (Plant Samples)

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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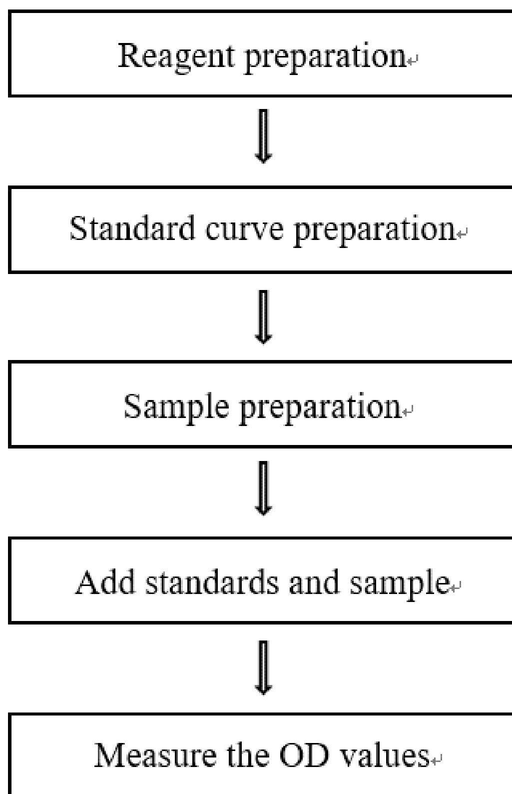
Assay summary



Intended use

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

Detection principle

Under alkaline conditions, tungsten-molybdenum acid can be reduced by phenols and produce blue compounds, which has a characteristic absorption peak at 760 nm. The content of total phenols in sample can be calculated indirectly by measuring the absorbance at 760 nm.

Kit components & storage

Item	Component	Size 1(96 T)	Size 2(500 Assays)	Storage
Reagent 1	Chromogenic Reagent	10 mL ×1 vial	50 mL ×1 vial	2-8℃, 12 months shading light
Reagent 2	Alkali Reagent	Powder ×1 vial	Powder ×5 vials	2-8℃, 12 months
Reagent 3	Standard	Powder ×2 vials	Powder ×10 vials	2-8℃, 12 months shading light
	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. 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:

Incubator, Vacuum drying oven, Centrifuge, Microplate reader (760 nm)

Reagents:

Double distilled water, 60% alcohol

Reagent preparation

① Equilibrate all the reagents to room temperature before use.

② The preparation of alkali working solution:

Dissolve a vial of alkali reagent with 10 mL of double distilled water. Store at 2~8 ℃ for a month protected from light.

③ The preparation of 1 mg/mL standard solution:

Dissolve a vial of standard with 10 mL of double distilled water. Store at 2~8 ℃ for a month protected from light.

④ 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 double distilled water to a serial concentration. The recommended dilution gradient is as follows: 150, 120, 100, 80, 60, 40, 20, 0 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 standard (μL)	0	20	40	60	80	100	120	150
Double distilled water (μL)	1000	980	960	940	920	900	880	850

Sample preparation

① Sample preparation

① Take fresh plant tissue (5-10 g), rinse the surface with distilled water and dry with filter paper. Then dry to constant weight in a vacuum drying oven at 80°C (The difference between the two weights should be less 0. 3 mg). Crush and seal at room temperature.

② Weigh 0.04 g crushed sample and add 1 mL of 60% alcohol. Homogenate for 60 s and centrifuge at 10000 g for 10 min at room temperature. 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 tissue homogenate	20-30
Daucus carota tissue homogenate	5-15
Spinacia oleracea tissue homogenate	15-25
Leek tissue homogenate	10-20

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 chromogenic reagent, stand at room temperature for 2 min before adding other reagents.

② After adding alkali working solution and double distilled water, stand at room temperature for 10 min before measuring the OD value.

Operating steps

① Standard well: add 10 μL of standard solution with different concentrations into the corresponding wells.

Sample well: add 10 μL of sample into the corresponding wells.

Control well: add 10 μL of sample into the corresponding wells.

② Add 50 μL of chromogenic reagent into the sample wells and standard wells.

Add 50 μL of double distilled water into the control wells.

③ Mix well for 5 s with microplate reader and stand at room temperature for 2 min.

④ Add 50 μL of alkali working solution and 90 μL of double distilled water into each well.

⑤ Mix well for 5 s with microplate reader and stand at room temperature for 10 min. Measure the OD values of each well at 760 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:

$$\frac{\text{Plant total phenol content}}{\text{(mg/g wet weight)}} = (\Delta A_{760} - b) \div a \times V \div m \div 1000^{*} \times f$$

[Note]

ΔA_{760}: $OD_{Sample} - OD_{Control}$.

V: the volume of added extraction solution, 1 mL.

m: weight of sample, 0.04 g.

1000*: 1000 μg = 1 mg.

f: dilution factor of sample before tested.

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)	5.70	35.40	106.20
%CV	4.5	4.2	3.6

Inter-assay Precision

Three human serum 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)	5.70	35.40	106.20
%CV	3.8	4.6	4.8

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 95%.

	Standard 1	Standard 2	Standard 3
Expected Conc. (μg/mL)	25	76	114
Observed Conc. (μg/mL)	23.5	75.2	104.9
Recovery rate (%)	94	99	92

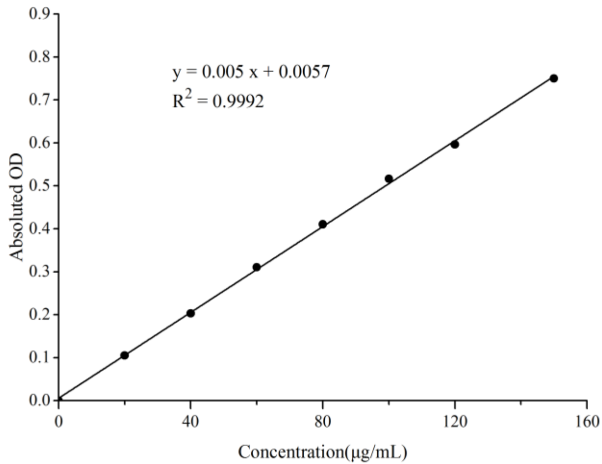
Sensitivity

The analytical sensitivity of the assay is 1.05 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	0.050	0.150	0.251	0.354	0.458	0.564	0.642	0.817
	0.050	0.161	0.255	0.367	0.462	0.569	0.650	0.783
Average OD	0.050	0.155	0.253	0.360	0.460	0.567	0.646	0.800
Absoluted OD	0.000	0.105	0.203	0.311	0.411	0.517	0.596	0.750



Appendix Π Example Analysis

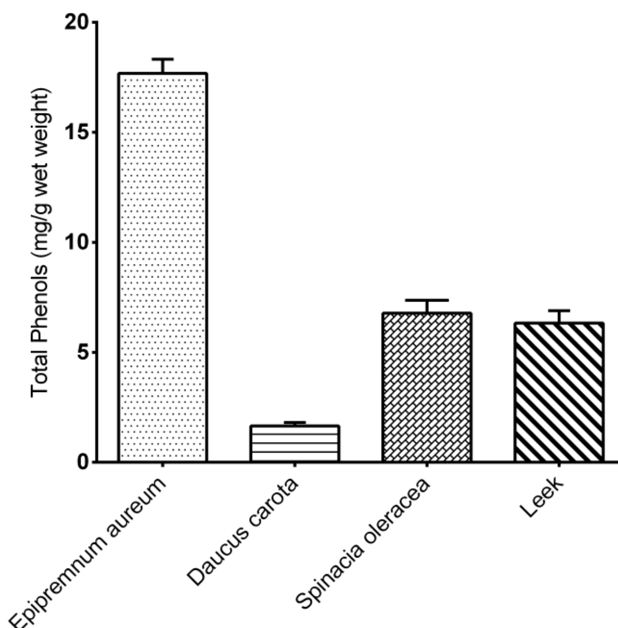
Example analysis：

Take 10 μL of epipremnum aureum tissue homogenate supernatant diluted for 30 times and carry the assay according to the operation steps. The results are as follows:

standard curve: y = 0.005 x+ 0.0057, the average OD value of the sample is 0.170, the average OD value of the control is 0.050, and the calculation result is:

Plant total phenol content (mg/g wet weight) = (0.17 0- 0.050 - 0.0057) ÷0.005 ×1 ÷0.04 ÷ 1000 ×20 = 11.43 mg/g wet weight

Detect epipremnum aureum tissue homogenate (dilute for 30 times), daucus carota tissue homogenate (dilute for 10 times), spinacia oleracea tissue homogenate (dilute for 20 times) and leek tissue homogenate (dilute for 20 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.