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

Catalog No：E-BC-K354-S

Specification：100 Assays(42 samples)/200Assays (92 samples)

Measuring instrument：Spectrophotometer（760 nm）

Detection range：0.73-150 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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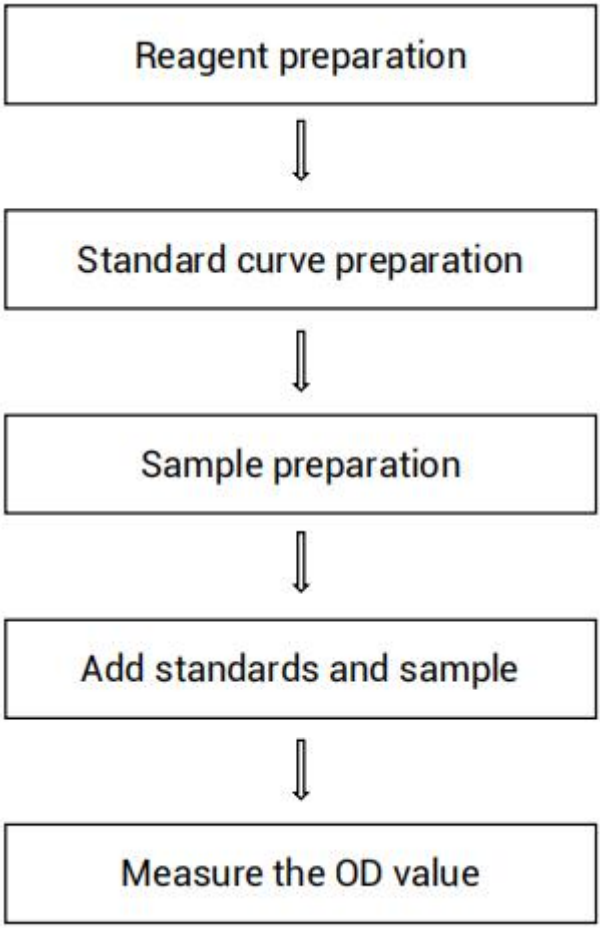
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Assay summary



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 (100 assays)	Size 2 (200 assays)	Storage
Reagent 1	Folin Phenol Reagent	60 mL × 1 vial	60 mL × 2 vials	2-8℃, 12 months shading light
Reagent 2	Alkali	Powder × 2 vials	Powder × 4 vials	2-8℃, 12 months
Reagent 3	O-dihydroxybenzene	10 mg × 4 vials	10 mg × 8 vials	2-8℃, 12 months shading light

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:

UV-visible Spectrophotometer (760 nm), Micropipettor, Vacuum dryer, Vortex mixer, Magnetic Stirrers, Ultrasonic cell grinder, crusher

Reagents:

Double distilled water, 60% Ethanol

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 double distilled water to a final volume of 50 mL. Store at 2~8°C for a month.

③ The preparation of 1 mg/mL o-dihydroxybenzene solution：

Dissolve a vial of O-dihydroxybenzene powder with double distilled water to a final volume of 10 mL. Store at 2~8°C 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 o-dihydroxybenzene solution with double distilled water to a serial concentration. The recommended dilution gradient is as follows: 0, 40, 60, 80, 100, 120, 150 μg/mL. Reference is as follows:

Item	①	②	③	④	⑤	⑥	⑦
Concentration (μg/mL)	**0**	**40**	**60**	**80**	**100**	**120**	**150**
1 mg/mL standard (μL)	0	40	60	80	100	120	150
Double distilled water (μL)	1000	960	940	920	900	880	850

Sample preparation

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 40℃ (The difference between the two weights should be less 0. 3 mg). Crush and screen with 40 mesh sieve, sealed at room temperature.

② Weigh 0.1 g crushed sample and add 2.5 mL of 60% ethanol (self-prepared). Treat the sample with sonication (power: 300W, 3 seconds/time, interval for 4 seconds, total: 30 min). Centrifuge at 10000 g for 10 min at 25℃. Take the supernatant for detection.

The key points of the assay

O-dihydroxybenzene standard solution should be prepared freshly, as it is easily oxidized.

Operating steps

① Standard tube: Take 0.1 mL of O-dihydroxybenzene with different concentrations into EP tubes.
Sample tube: Take 0.1 mL of pretreated sample into EP tubes.
Control tube: Take 0.1 mL of pretreated sample into EP tubes.

② Add 0.5 mL of folin phenol reagent into standard tubes and sample tubes, oscillate fully with a vortex mixer and standard at room temperature for 2 min.

③ Add 0.5 mL of alkali application solution, 0.9 mL of double distilled water into standard tubes and sample tubes, add 0.5 mL of alkali application solution, 1.4 mL of double distilled water into control tubes.

④ Oscillate fully with a vortex mixer and stand for 10 min at room temperature. Set the spectrophotometer to zero with double distilled water and measure the absorbance values of each tube at 760 nm with 0.5 cm optical path cuvette.

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:

$$\text{Total phenols content (mg/g tissue)} = (\Delta A_{760} - b) \div a \times V \div W \div 1000^{*} \times f$$

[Note]

ΔA_{760}: Absolute OD ($OD_{Sample} - OD_{Control}$)

V: the volume of added extraction solution, 2.5 mL of 60% ethanol.

W: Weight of sample, 0.1 g

*: Unit conversion, 1000 μg=1 mg.

f: Dilution factor of sample before test.

Appendix I Performance Characteristics

1. Parameter:

Intra-assay Precision

Three chrysanthemum tea 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 (mg/g tissue)	9.40	48.50	96.20
%CV	2.3	1.8	1.6

Inter-assay Precision

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

Parameters	Sample 1	Sample 2	Sample 3
Mean (mg/g tissue)	9.40	48.50	96.20
%CV	2.1	2.6	2.8

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 101%.

	Standard 1	Standard 2	Standard 3
Expected Conc. (μg/mL)	32.5	84.5	126
Observed Conc. (μg/mL)	32.2	87.0	127.3
Recovery rate (%)	99	103	101

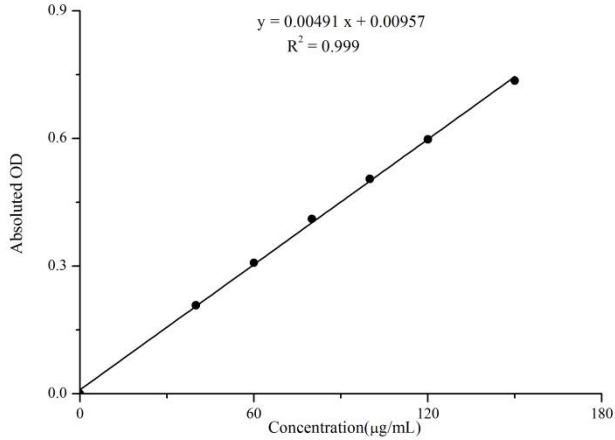
Sensitivity

The analytical sensitivity of the assay is 0.73 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**	**40**	**60**	**80**	**100**	**120**	**150**
Average OD	0.001	0.209	0.309	0.412	0.506	0.599	0.737
Absoluted OD	0	0.208	0.308	0.411	0.505	0.598	0.736



Appendix Π Example Analysis

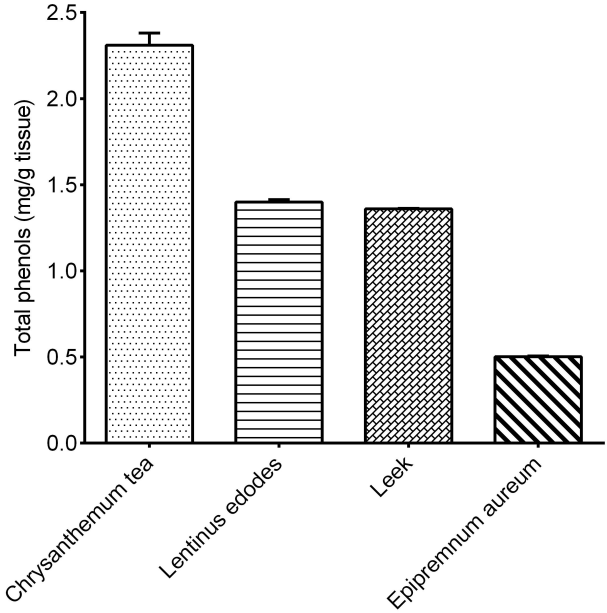
Example analysis：

Take 0.1 mL of lentinus edodes supernatant and carry the assay according to the operation steps. The results are as follows:

Standard curve: y = 0.00514 x + 0.00525 (R^2=0.99723), the average OD value of the sample is 0.288, the average OD value of the control is 0.003, and the calculation result is:

Total phenols content (mg/g tissue) = (0.288 - 0.003 - 0.00525) ÷ 0.00514 × 5 ÷ 0.2 ÷ 1000 = 1.361 mg/g tissue

Detect chrysanthemum tea, lentinus edodes, leek, epipremnum aureum 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.