Total Phenols Colorimetric Assay Kit (Plant Samples)

Catalog No: E-BC-K354-S

Method: Colorimetric method

Specification: 100 Assays (Can detect 42 samples without duplication)

Instrument: Spectrophotometer

Sensitivity: 0.73 μg/mL

Detection range: 0.73-150 μg/mL

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

General information

▲Intended use

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

▲Background

Plant total phenol is a common secondary natural metabolite in plants. There are several kinds of phenolic compounds, such as hydroxybenzoic acid, hydroxy cinnamic acid, flavonoids, chalcone, flavonoids, lignin, coumarin and astragalus. Phenolic compounds are antioxidants that delay or prevent oxidation and oxygen radical reactions.

▲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	Specification	Storage
Reagent 1	Folin Phenol Reagent	60 mL × 1 vial	2-8°C , 6 months, shading light
Reagent 2	Alkali	Powder × 2 vials	2-8°C , 6 months
Reagent 3	O-dihydroxybenzene	Powder × 4 vials	2-8°C , 6 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.			

▲Materials prepared by users

Instruments

Spectrophotometer (760 nm), Micropipettor, Vacuum dryer, Vortex mixer, Ultrasonic cell grinder, Crusher

Consumptive material

Tips (10 μL, 200 μL, 1000 μL), EP tubes (10 mL, 2 mL)

Reagents:

Double distilled water, 60% Ethanol

▲Safety data

Some of the reagents in the kit contain dangerous substances. It should be avoided to touch the skin and clothing. Wash immediately with plenty of water if touching it carelessly. All the samples and waste material should be treated according to the relevant rules of laboratory's biosafety.

▲Precautions

Before the experiment, please read the instructions carefully, and wear gloves and work clothes.

▲The key point of the assay

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

Pre-assay preparation

▲ Reagent preparation

1. Preparation of reagent 2 application solution:
   Dissolve a vial of reagent 2 powder with double distilled water to a final volume of 50 mL. The prepared solution can be stored at 2-8°C for a month.
2. Preparation of 1 mg/mL O-dihydroxybenzene solution:
   Dissolve a vial of reagent 3 powder with double distilled water to a final volume of 10 mL. The prepared solution can be stored at 2-8°C for a month with shading light.

▲ Sample preparation

1. Drying and crushing of plant tissue:

   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. Extraction of plant tissue:

   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.

▲Dilution of sample

It is recommended to take 2~3 samples with expected large difference to do pre-experiment before formal experiment and dilute the sample according to the result of the pre-experiment and the detection range (0.73-150 μg/mL).

Assay protocol	
Ambient temperature	25-30°C
Optimum detection wavelength	760 nm

Instructions for the use of transferpettor:

(1) Equilibrate the pipette tip in that reagent before pipetting each reagent.

(2) Don't add the liquid outside the tips into the reaction system when pipetting each reagent.

Assay protocol

▲Operating steps

The preparation of standard curve

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.

The measurement of samples

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

(2) Add 0.5 mL of reagent 1 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 reagent 2 application solution, 0.9 mL of double distilled water into standard tubes and sample tubes, add 0.5 mL of reagent 2 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.

▲Operation table

	Control tube	Standard tube	Sample tube
Sample (mL)	0.1		0.1
O-dihydroxybenzene with different concentration (mL)		0.1	
Reagent 1 (mL)		0.5	0.5
Oscillate fully with a vortex mixer and standard at room temperature for 2 min.			
Reagent 2 application solution (mL)	0.5	0.5	0.5
Double distilled water (mL)	1.4	0.9	0.9
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

Plot the standard curve by using OD value of standard and correspondent concentration as y-axis and x-axis respectively. Create the standard curve with graph software (or EXCEL). The concentration of the sample can be calculated according to the formula based on the OD value of sample. The standard curve is: y = ax + b.

Total phenols content (mg/g tissue) = (ΔA_{760} - b) ÷ a ×V ÷ W ÷ 1000* × f

Note:

y: $OD_{Standard}$ – OD_{Blank}

x: The concentration of standard

a: The slope of standard curve

b: The intercept of standard curve

ΔA_{760}: 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.

Notes

1. This kit is for research use only.
2. Instructions should be followed strictly, changes of operation may result in unreliable results.
3. The validity of kit is 6 months.
4. Do not use components from different batches of kit.

Appendix I Performance characteristics

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Detection range	0.73-150 μg/mL	Average intra-assay CV (%)	1.9
Sensitivity	0.73 μg/mL	Average inter-assay CV (%)	2.5
Average recovery rate (%)	101		

▲Example analysis

Take 0.1 mL of lentinus edodes supernatant and carry the assay according to the operation table.

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