Technical Manual

Total Phenols Colorimetric Assay Kit (Plant Samples)

- Catalogue Code: MAES0184
- Size: 100 Assays
- Research Use Only

1. Key Features and Sample Types

Detection method:

Colorimetric method

Specification:

100 Assays

Range:

0.73-150 µg/mL

Sensitivity:

0.73 µg/mL

Storage:

2-8°C for 6 months

Expiry:

See Kit Label

Experiment Notes:

This kit is for **research use only.**

Instructions should be strictly followed. Changes of operation may result in unreliable results.

The validity of kit is 6 months.

Do not use components from different batches of kit.

2. 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.

3. Intended Use

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

4. 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.

5. Kit Components & Storage

Item	Specification	Storage
Folin Phenol Reagent	60 mL × 1 vial	2-8°C, 6 months, avoid direct sunlight
Alkali	Lyophilized × 2 vials	2-8°C, 6 months
O-dihydroxybenzene	10 mg × 4 vials	2-8°C, 6 months, avoid direct sunlight

Materials required but not supplied

- Micropipettor
- Vacuum dryer
- Vortex mixer
- Magnetic Stirrers
- Ultrasonic cell grinder
- Crusher
- UV-visible Spectrophotometer (760 nm)
- Tips (10 µL, 200 µL, 1000 µL)
- EP tubes (10 mL, 2 mL)
- Double distilled water
- 60% Ethanol

6. Assay Notes:

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

7. Reagent Preparation:

1. Bring all the reagents to room temperature before use.

2. Preparation of **Alkali application solution:** Dissolve a vial of alkali 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.

3. Preparation of **o-dihydroxybenzene solution (1 mg/mL):** Dissolve a vial of O-dihydroxybenzene powder with double distilled water to a final volume of 10 mL. The prepared solution can be stored at 4°C for a month and avoid direct sunlight.

8. 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°C (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°C. Take the supernatant for detection.

Sample Notes:

The concentration should be determined before preforming the assay. If the sample concentration is not within the range of the standard curve, users must determine the optimal sample dilutions for their particular experiments.

If the sample type is not included in the manual, a preliminary experiment is suggested to verify the validity.

Dilution of Samples:

Large variances in results may be seen when performing pre-experiments. Dilute the sample according to the result of the pre-experiment and the detection range (0.73-150 μg/mL).

9. Assay Protocol

Ambient Temperature: 25-30°C

Optimum detection wavelength: 760 nm

10. Operation 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 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.

Operation Table

	Control tube	Standard tube	Sample tube
Sample (mL)	0.1		0.1
O-dihydroxybenzene with different concentration (mL)		0.1	
Folin phenol reagent (mL)		0.5	0.5
Oscillate fully with a vortex mixer and standard at room temperature for 2 min.			
Alkali 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.			

11. Calculations

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.

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

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;
Δ_{A760}: $OD_{Sample} - OD_{Control}$;
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.

12. Performance Characteristics

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

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 (R2=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:

$$\textbf{Total phenols content mg/g tissue} = \frac{(0.288 - 0.003 - 0.00525)}{0.00514} \times 5 \div 0.2 \div 1000$$

$$= 1.361 \text{ mg/g tissue}$$

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Safety Notes

Some of the reagents in the kit contain dangerous substances. Prevent touching 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.

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

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