Technical Manual

Total Phenols Colorimetric Assay Kit (Plant samples)

- **Catalogue Code: MAES0183**
- **Size: 96T**
- **Research Use Only**

1. Key Features and Sample Types

Detection method:

Colorimetric method

Specification:

96T

Range:

1.05-148 µg/mL

Sensitivity:

1.05 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
Chromogenic Reagent	5 mL × 2 vials	2-8°C, 6 months, avoid direct sunlight
Alkali Reagent	Lyophilized × 2 vials	2-8°C, 6 months
Standard	10 mg × 2vials	2-8°C, 6 months, avoid direct sunlight
Microplate	96 wells	No requirement
Plate Sealer	2 pieces	

Materials required but not supplied

- Micropipettor
- Incubator
- Vacuum drying oven
- Centrifuge
- Microplate reader (760 nm)
- Tips (10 μL, 200 μL, 1000 μL)
- EP tubes (1.5 mL, 2 mL)
- Double distilled water
- 60% alcohol

6. Assay Notes:

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.

7. Reagent Preparation:

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

2. Preparation of **Alkali working solution:** Dissolve a vial of alkali reagent with 10 mL of double distilled water. The prepared solution can be stored at 2-8°C for a month.

3. Preparation of **standard solution (1 mg/mL):** Dissolve a vial of standard with 10 mL of double distilled water. The prepared solution can be stored at 2-8°C and avoid direct sunlight for a month.

8. Sample Preparation

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.

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.

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 (1.05-148 μg/mL).

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.

9. Assay Protocol

Ambient Temperature: 25-30°C

Optimum detection wavelength: 760 nm

Plate Set Up:

	1	2	3	4	5	6	7	8	9	10	11	12
A	A	A	S1	S1'	S9	S9'	S17	S17'	S25	S25'	S33	S33'
B	B	B	S2	S2'	S10	S10'	S18	S18'	S26	S26'	S34	S34'
C	C	C	S3	S3'	S11	S11'	S19	S19'	S27	S27'	S35	S35'
D	D	D	S4	S4'	S12	S12'	S20	S20'	S28	S28'	S36	S36'
E	E	E	S5	S5'	S13	S13'	S21	S21'	S29	S29'	S37	S37'
F	F	F	S6	S6'	S14	S14'	S22	S22'	S30	S30'	S38	S38'
G	G	G	S7	S7'	S15	S15'	S23	S23'	S31	S31'	S39	S39'
H	H	H	S8	S8'	S16	S16'	S24	S24'	S32	S32'	S40	S40'

Note: A-H, standard wells; S1-S40, sample wells; S1'-S40', control wells.

10. Operation Steps

The preparation of standard curve

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.

The measurement of samples

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

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

4. Mix fully for 5 s with microplate reader and stand at room temperature for 2 min.

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

6. Mix fully 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.

Operation Table

	Standard well	Sample well	Control well
Standards solution with different concentrations (μL)	10		
Sample (μL)		10	10
Chromogenic reagent (μL)	50	50	
Double distilled water (μL)			50
Mix fully for 5 s with microplate reader and stand at room temperature for 2 min.			
Alkali working solution (μL)	50	50	50
Double distilled water (μL)	90	90	90
Mix fully 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.			

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.

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

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, 1 mL.
m: Weight of sample, 0.04 g
***:** Unit conversion, 1000 μg=1 mg.
f: Dilution factor of sample before test.

12. Performance Characteristics

Detection Range	1.05-148 μg/mL
Sensitivity	1.05 μg/mL
Average recovery rate (%)	95
Average inter-assay CV (%)	4.4
Average intra-assay CV (%)	4.1

Analysis

Take 10 μL of epipremnum aureum tissue homogenate supernatant diluted for 30 times and carry the assay according to the operation table.

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:

$$\textbf{Total phenols content mg/g wet weight} = \frac{(0.170 - 0.050 - 0.0057)}{0.005} \times 1 \div 0.04 \div 1000 \times 20$$

$$= 11.43 \text{ mg/g wet weight}$$

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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