Total Phenols Colorimetric Assay Kit (Plant Samples)

Catalog No: E-BC-K354-M

Method: Colorimetric method

Specification: 96T (Can detect 40 samples without duplication)

Instrument: Microplate reader

Sensitivity: 1.05 μg/mL

Detection range: 1.05-148 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	Chromogenic Reagent	10 mL × 1 vial	2-8℃ , 6 months, shading light
Reagent 2	Alkali Reagent	Powder × 1 vials	2-8℃ , 6 months
Reagent 3	Standard	Powder × 2 vials	2-8℃ , 6 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.			

▲Materials prepared by users

Instruments

Incubator, Vacuum drying oven, Centrifuge, Microplate reader (750-770 nm)

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

1. After adding reagent 1, stand at room temperature for 2 min before adding other reagents.
2. After adding reagent 2 working solution and double distilled water, stand at room temperature for 10 min before measuring the OD value.

Pre-assay preparation

▲ Reagent preparation

1. Bring all reagents to room temperature before use.
2. Preparation of reagent 2 application solution:
   Dissolve a vial of reagent 2 with 10 mL of double distilled water. The prepared solution can be stored at 2-8°C for a month
2. Preparation of 1 mg/mL standard solution:
   Dissolve a vial of reagent 3 with 10 mL of double distilled water. The prepared solution can be stored at 2-8°C with shading light for a month .

▲ 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.

▲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 (1.05-148 μg/mL).

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.

Assay protocol	
Ambient temperature	25-30℃
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

▲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.

▲Operating 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 reagent 1 into the sample wells and standard wells. Add 50 μL of double distilled water into the control wells.
3. Mix fully for 5 s with microplate reader and stand at room temperature for 2 min.
4. Add 50 μL of reagent 2 working solution and 90 μL of double distilled water into each well.
5. 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

	Sample well	Standard well	Control well
Standards solution with different concentrations (μL)	10		
Sample (μL)		10	10
Reagent 1 (μL)	50	50	
Double distilled water (μL)			50
Mix fully for 5 s with microplate reader and stand at room temperature for 2 min.			
Reagent 2 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.			

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 wet weight) = (ΔA_{760} - b) ÷ a ×V ÷ W ÷ 1000* × f

Note:

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;

ΔA_{760}: OD_{Sample} – $OD_{Control}$;

V: the volume of added extraction solution, 1 mL of 60% ethanol;

W: Weight of sample, 0.04 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	1.05-148 μg/mL	Average intra-assay CV (%)	4.1
Sensitivity	1.05 μg/mL	Average inter-assay CV (%)	4.4
Average recovery rate (%)	95		

▲Example 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:

Total phenols content (mg/g wet weight)
= (0.170-0.050-0.0057)÷0.005×1÷0.04÷1000×20
= 11.43 mg/g wet weight