TECHNICAL MANUAL

Total Phenols Colorimetric Assay Kit (Plant samples)

- **SKU CODE:** MAES0183
- **SIZE:** 96 Tests
- **DETECTION PRINCIPLE:** Assay Kit
- **RUO:** Research-Use-Only

Revised: 01/2026

1. Assay summary

- Reagent preparation
- Standard curve preparation
- Sample preparation
- Add standards and samples
- Incubate and measure absorbance
- Calculate total phenol content

2. Intended use

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

3. Detection principle

Under alkaline conditions, tungsten-molybdenum acid can be reduced by phenols and produce blue compounds, which have a characteristic absorption peak at 760 nm. The content of total phenols in the sample can be calculated indirectly by measuring the absorbance at 760 nm.

4. Kit components & storage

Item	Component	Size 1(96 T)	Size 2(500 Assays)	Storage
Reagent 1	Chromogenic Reagent	10 mL × 1 vial	50 mL × 1 vial	2-8°C, 12 months, protect from light
Reagent 2	Alkali Reagent	Powder × 1 vial	Powder × 5 vials	2-8°C, 12 months
Reagent 3	Standard	Powder × 2 vials	Powder × 10 vials	2-8°C, 12 months, protect from light
	Microplate	96 wells		No requirement
	Plate Sealer	2 pieces		

5. Materials prepared by users

Instruments:

Incubator, Vacuum drying oven, Centrifuge, Microplate reader (760 nm)

Reagents:

Double distilled water, 60% alcohol

6. Reagent preparation

1. Equilibrate all reagents to room temperature before use.
2. **Preparation of alkali working solution:** Dissolve one vial of alkali reagent with 10 mL of double distilled water. Store at 2-8°C for one month, protected from light.
3. **Preparation of 1 mg/mL standard solution:** Dissolve one vial of standard with 10 mL of double distilled water. Store at 2-8°C for one month, protected from light.
4. **Preparation of standard curve:** Always prepare a fresh set of standards. Discard working standard dilutions after use. 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.

7. Standard curve preparation

Item	Concentration (μg/mL)	1 mg/mL standard (μL)	Double distilled water (μL)
①	0	0	1000
②	20	20	980
③	40	40	960
④	60	60	940
⑤	80	80	920
⑥	100	100	900
⑦	120	120	880
⑧	150	150	850

8. Sample preparation

1. **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 two weights should be less than 0.3 mg). Crush and seal at room temperature. Weigh 0.04 g crushed sample and add 1 mL of 60% alcohol. Homogenize for 60 s and centrifuge at 10,000 × g for 10 min at room temperature. Take the supernatant for detection.
2. **Dilution of sample:** The recommended dilution factor for different samples is as follows (for reference only): 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. For the dilution of other sample types, please perform a pre-test to confirm the dilution factor.

9. The key points of the assay

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

10. Operating steps

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. Add 50 μL of double distilled water into the control wells.
3. Mix well for 5 s with microplate reader and incubate at room temperature for 2 min.
4. Add 50 μL of alkali working solution and 90 μL of double distilled water into each well.
5. Mix well for 5 s with microplate reader and incubate at room temperature for 10 min. Measure the OD values of each well at 760 nm with microplate reader.

11. 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 absolute OD value.

3. Plot the standard curve by using absolute OD value of standard and corresponding concentration as y-axis and x-axis respectively. Create the standard curve (y = ax + b) with graph software (or Excel).

The sample:

Plant total phenol content (mg/g wet weight) = (ΔA_760 - b) ÷ a × V ÷ m ÷ 1000* × f

[Note]

ΔA_760: $OD_{Sample} - OD_{Control}$.

V: the volume of added extraction solution, 1 mL.

m: weight of sample, 0.04 g.

1000*: 1000 μg = 1 mg.

f: dilution factor of sample before tested.

12. Appendix I Performance Characteristics

Parameter:

Intra-assay Precision

Three human serum samples were assayed in replicates of 20 to determine precision within an assay. (CV = Coefficient of Variation)

Intra-assay Precision

Parameters	Sample 1	Sample 2	Sample 3
Mean (μg/mL)	5.70	35.40	106.20
%CV	4.5	4.2	3.6

Inter-assay Precision

Three human serum samples were assayed 20 times in duplicate by three operators to determine precision between assays.

Inter-assay Precision

Parameters	Sample 1	Sample 2	Sample 3
Mean (µg/mL)	5.70	35.40	106.20
%CV	3.8	4.6	4.8

Recovery

Take three samples of high concentration, middle concentration and low concentration to test the samples of each concentration for 6 times in parallel to get the average recovery rate of 95%.

Recovery

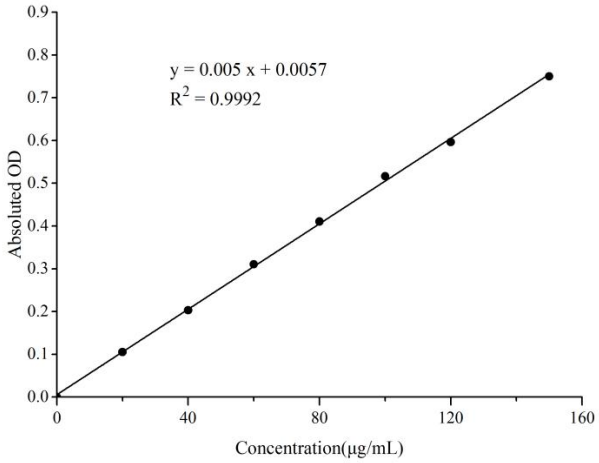
Standard	Expected Conc. (µg/mL)	Observed Conc. (µg/mL)	Recovery rate (%)
Standard 1	25	23.5	94
Standard 2	76	75.2	99
Standard 3	114	104.9	92

Sensitivity

The analytical sensitivity of the assay is 1.05 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.

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), the standard curve and data are provided below for reference only:



Standard curve data

Concentration (μg/mL)	OD value 1	OD value 2	Average OD	Absolute OD
0	0.050	0.050	0.050	0.000
20	0.150	0.161	0.155	0.105
40	0.251	0.255	0.253	0.203
60	0.354	0.367	0.360	0.311
80	0.458	0.462	0.460	0.411
100	0.564	0.569	0.567	0.517
120	0.642	0.650	0.646	0.596
150	0.817	0.783	0.800	0.750

13. Appendix II Example Analysis

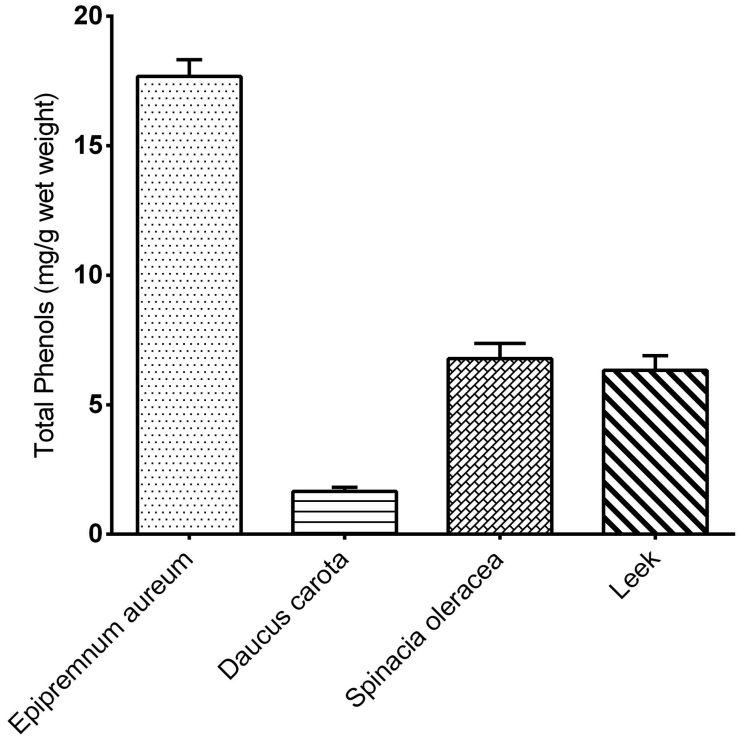
Example analysis:

Take 10 μL of epipremnum aureum tissue homogenate supernatant diluted 30 times and carry out the assay according to the operation steps. The results are as follows:

Standard curve: y = 0.005x + 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:

Plant total phenol content (mg/g wet weight) = (0.170 - 0.050 - 0.0057) ÷ 0.005 × 1 ÷ 0.04 ÷ 1000 × 30 = 11.43 mg/g wet weight

Detect epipremnum aureum tissue homogenate (diluted 30 times), daucus carota tissue homogenate (diluted 10 times), spinacia oleracea tissue homogenate (diluted 20 times) and leek tissue homogenate (diluted 20 times) according to the protocol. The results are as follows:



14. Statement

1. This assay kit is for Research Use Only. Assay Genie assumes no responsibility for any problems or legal liabilities arising from the use of this kit for clinical diagnosis or any other purpose.
2. Please read the instructions carefully and calibrate the instruments before performing the experiments. Follow the instructions strictly throughout the procedure.
3. Protective measures must be taken, including wearing a lab coat and latex gloves.
4. If the concentration of the substance is not within the detection range, perform an additional dilution or concentration step on the sample.
5. It is recommended to perform a pre-test if your sample type is not listed in the instruction manual.

6. Experimental results are closely related to reagent quality, operator technique, environmental conditions, and other factors. Assay Genie guarantees the quality of the kits only and is NOT responsible for sample consumption resulting from use of the assay kits. It is advisable to estimate the expected sample usage and reserve sufficient samples before starting the experiment.

Assay Genie 100% money-back guarantee!

If you are not satisfied with the quality of our products and our technical team cannot resolve your problem, we will give you 100% of your money back.

Manufacturers Statement: This final kit system is assembled and quality-released by Assay Genie Limited.