Plant Total Phenol (TP)Assay Kit

Note: Before the experiment, it is recommended to select 2-3 sample with large expected differences for pre-experiment.

Operation Equipment: Spectrophotometer/microplate reader

Cat No: BC1345

Size: 100T/48S

Product Composition: Before use, please carefully check whether the volume of the reagent is consistent with the volume in the bottle. If you have any questions, please contact Solarbio staff in time.

Reagent name	Size	Storage
Extract Solution	Solution 125 mL×1 (Required but not provided)	RT
Reagent I	Solution 5 mL×1	2-8℃
Reagent II	Solution 8 mL×1	2-8℃
Standard	Powder×1	2-8℃

Solution preparation:

1. **Extract solution:** 60% alcohol (V: V), self-provided reagent, store at room temperature.
2. **Standard:** 5 mg of gallic acid. Before use, add 1 mL of distilled water, heat it at 50℃ and dissolve it to prepare 5mg/mL standard solution. It could be stored at 2-8℃ for two weeks.

Description:

Plant phenols have the function of scavenging free radicals, anti-oxidation and anti-aging. It is widely used in cosmetics, food, medicine and other fields because of its high nutritional value and health care function.

In alkaline conditions, phenolic substance reduce tungsten-molybdic acid to form blue compounds which has an absorption peak at 760 nm. The total phenol content of the sample is obtained by measuring the absorbance at 760nm.

Technical Index:

Minimum detection limit: 0.0015 mg/mL

linear range: 0.0024-0.3125 mg/mL

Required but not provided:

Spectrophotometer/microplate reader, micro glass cuvette/96 well plate, balance, oven, crusher/mortar, 30-50 mesh sieve, ultrasonic cleaner, centrifuge, 60% alcohol, distilled water.

Procedure:

I. Total phenol extraction:

Dry the sample to constant weight, smash. After screening with the 30-50 mesh sieve, add 2.5 mL of

Extract solution to 0.1 g of tissue and extract by ultrasonic cleaner (power 300W, 60℃ for 30 min). centrifuge at 12000 rpm for 10 min at 25℃.

II. Determination procedure.

1. Preheat spectrophotometer/microplate reader for 30 min, adjust wavelength to 760 nm, set spectrophotometer counter to zero with distilled water.
2. Dilution of standard solution: dilute the standard with distilled water to 0.1562, 0.0781, 0.0391, 0.0195, 0.0098, 0.0049mg/mL standard solution.
3. Standard solution dilution can refer to the following table:

Number	Pre dilution concentration (mg/mL)	Standard liquid volume (μL)	Volume of standard dilution solution (μL)	Diluted concentration (mg/mL)
1	5	125	875	0.625
2	0.625	250	750	0.15625
3	0.15625	200	200	0.078125
4	0.078125	200	200	0.0391
5	0.0391	200	200	0.0191
6	0.0191	200	200	0.0098
7	0.0098	200	200	0.0049

Note: Each standard tube in the following experiment requires 10 μL of standard solution (be careful not to directly test the absorbance of the standard solution in this step).

4. Operation table.

Reagent (μL)	Control tube (A_C)	Test tube (A_T)	Standard tube (A_S)	Blank tube (A_B)
Sample	10	10	-	-
Standard	-	-	10	-
Distilled water	-	-	-	10
Reagent I	-	50	50	50
Mix thoroughly, incubate at room temperature for 2 min.				
Reagent II	50	50	50	50
Distilled water	140	90	90	90
Mix thoroughly, incubate at room temperature for 10 min. Detect the absorbance of 760 nm in micro glass cuvette or 96 well plate, and record it as A_C, A_T, A_S and A_B, and calculate $\Delta A_S = A_S - A_B$, $\Delta A_T = A_T - A_C$. Each test tube should be provided with one contrast tube. Standard curve and blank tube only need to be measured once or twice.				

III. Calculation.

1. Standard curve drawing.

   Taking the concentration of each standard solution as the x-axis and its corresponding ΔA_S as the y-axis, draw a standard curve to get the standard equation $y = kx + b$, and bring ΔA_T into the equation

to get x (mg/mL).

2. Calculation of plant total phenol

a. Sample weight

Total phenol (mg/g weight) = $x \times V_E \div W = 2.5x \div W$

b. Protein concentration

Total phenol (mg/mg prot) = $x \times V_E \div (Cpr \times V_E) = x \div Cpr$

V_E: Extract solution volume; 2.5 mL;

W: Sample weight, g;

Cpr: Protein concentration, mg/mL.

Note:

1. If the measured absorbance value exceeds the absorbance value in the linear range, you can increase the sample volume or dilute the sample before performing the measurement.
2. Reagent I have a certain irritation to the skin, please take precautions during operation.

Examples:

1. Add 0.1g treated purple flower to 2.5mL extract solution, after treating sample follow the determination procedure to operate, with a 96 well plate to calculate: $\Delta A = A_T - A_B = 0.506 - 0.041 = 0.465$, standard curve: $y = 3.083x + 0.01$, calculate $x = 0.1476$, according with weight of sample to calculate:

   Total phenol (mg/g weight) = $2.5x \div W = 2.5 \times 0.1476 \div 0.1 = 3.69$ mg/g weight.

Recent Product citations:

[1] Yang R, Chen X, Zhang D, Wang H, Zhou W, Lin W, Qi Z. Steam-Exploded Pruning Waste as Peat Substitute: Physiochemical Properties, Phytotoxicity and Their Implications for Plant Cultivation. Int J Environ Res Public Health. 2022 Apr 27;19(9):5328. doi: 10.3390/ijerph19095328. PMID: 35564722; PMCID: PMC9103252.

[2] Lei Y, Wang W, Zhang C, Wang D, Zhuang W, Zheng B, Lo YM, Tian Y. Evaluation of the chemical qualities and microstructural changes of Lentinula edodes caused by airborne ultrasonic treatment combined with microwave vacuum drying. J Food Sci. 2021 Mar;86(3):667-676. doi: 10.1111/1750-3841.15593. Epub 2021 Jan 26. PMID: 33496977.

[3] Wu Y, Xu X, Jiang X, Liu S, Lin J, Lin X, Zhang Y, Shi C, Zhao C, Yang J. Application of polysaccharide-rich solution derived from waste macroalgae Enteromorpha prolifera in cherry tomato preservation and utilizing post-extraction residue for crude bio-oil production. Food Chem. 2023 May 30;409:135301. doi: 10.1016/j.foodchem.2022.135301. Epub 2022 Dec 24. PMID: 36587516.

[4] Jiao X, Li F, Zhao J, Wei Y, Zhang L, Wang H, Yu W, Li Q. Structural diversity and physicochemical properties of polysaccharides isolated from pumpkin (Cucurbita moschata) by

different methods. Food Res Int. 2023 Jan;163:112157. doi: 10.1016/j.foodres.2022.112157. Epub 2022 Nov 19.

PMID: 36596108.

[5] Ren G, Yang P, Cui J, Gao Y, Yin C, Bai Y, Zhao D, Chang J. Multiomics Analyses of Two Sorghum Cultivars Reveal the Molecular Mechanism of Salt Tolerance. Front Plant Sci. 2022 May 23;13:886805. doi: 10.3389/fpls.2022.886805. PMID: 35677242; PMCID: PMC9168679.

Reference:

[1] Maryam Akhbari, Sepideh Hamedi, Zahra-sadat Aghamiri. Optimization of total phenol and anthocyanin extraction from the peels of eggplant (Solanum melongena L.) and biological activity of the extracts [J]. Journal of Food Measurement and Characterization, 2019, 13(4): 29-37.

Related Products:

BC1300/BC1305	Ceruloplasmin (CP) Assay Kit
BC1310/BC1315	Total antioxidant capacity (T-AOC) Assay Kit
BC1370/BC1375	Total Sulfhydryl Assay Kit