

Plant Total Phenol (TP) Assay Kit

Note: Take two or three different samples for prediction before test.

Operation Equipment: Spectrophotometer

Cat No: AK0450

Size:50T/24S

Components:

Extract solution: 60% alcohol (V:V), self-provided reagent.

Reagent I: Liquid 20 mL×1, store at 4°C .

Reagent II: Liquid 25 mL×1, store at 4°C .

Standard: Powder×1, store at 4°C . 5 mg of gallic acid. Before use, add 1 mL of distilled water, heat it at 50°C and dissolve it to prepare 5mg/mL standard solution.

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 a absorption peak at 760 nm. The total phenol content of the sample is obtained by measuring the absorbance at 760nm.

Required but not provided:

Balance, oven, crusher, sieve, ultrasonic breaker, centrifuge, 60% alcohol, spectrophotometer, 1 mL glass cuvette, distilled water.

Procedure:

I. Total phenol extraction:

Dry the sample to constant weight, smash. After screening with the 40 mesh sieve, add 2.5 mL of Extract solution to 0.1 g of tissue and extract by ultrasonic breaker, (power 300W, crush 5s, interval 8s, 60C for 30 min). centrifuge at 12000 rpm for 10 min at 25C. Take supernatant and make the liquid to a volume of 2.5 mL with the Extract solution.

II. Preparation of standard.

The standard solution of 5 mg/mL standard solution is diluted to 0. 15, 0.078125, 0.039, 0.02, 0.01, 0.005, 0.0024 mg/mL for test.

III. Determination procedure.

1. Preheat spectrophotometer for 30 min, adjust wavelength to 760 nm, set zero with distilled water.
2. Add reagents according to the following table.

Reagent Name (μL)	Control tube (A _C)	Test tube (A _T)	Standard tube (A _S)	Blank tube (A _B)
Sample	50	50		-

Standard			50	
Distilled water				50
Reagent I	-	250	250	250
Mix thoroughly , incubate at room temperature for 2 min.				
Reagent II	250	250	250	250
Distilled water	700	450	450	450
Mix thoroughly, incubate at room temperature for 10 min. Use 1 mL glass cuvette, detect the absorbance at 760 nm.				

Note: Blank tube just test once or twice.

IV. Calculation.

1. Draw of standard curve.

With the concentration of different standard solution as x-axis, $\Delta A(A_S - A_B)$ as y-axis, draw standard curve $y=kx+b$. Bring $\Delta A=A_T - A_C$ to standard curve, calculate x (mg/mL).

2. Calculation of plant total phenol

a. Sample weight

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

b. Protein concentration

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

V_E : Extract solution volume; 2.5 mL;

W: Sample weight, g;

Cpr: Protein concentration , mg/mL.

Note:

1. If $OD > 1$, determine after diluting, multiply dilution multiple in equation.
2. Reagent I have a certain irritation to the skin, please take precautions during operation.

Examples:

1. Add 0.1g treated grape peel to 1mL extract solution, use ultrasonic wave to crack, with 300w at 60C, break for 5s and interrupt for 8s, 30min for whole process, centrifuge with 12000rpm at 25C for 10min, take supernatant and add extract solution to 1ml, follow the determination procedure to operate, and calculate: $\Delta A = A(T) - A(B) = 0.365 - 0.116 = 0.249$, standard curve: $y = 0.3144x + 0.0009$, calculate $x = 0.789$, according with mass of sample to calculate: Flavonoid content ($\mu\text{mol/g mass}$) $= x \div W = 0.789 \div 0.1 = 7.89 \text{ mg/g mass}$.

Examples:

1. Add 0.1g treated yellow flower to 2.5mL extract solution, after treating sample follow the determination procedure to operate, and calculate: $\Delta A = A(T) - A(B) = 0.679 - 0.000 = 0.679$, standard curve: $y = 5.5245x + 0.0102$, calculate $x = 0.1211$, according with mass of sample to calculate: Total phenol (mg/mg mass) $= 2.5x \div W = 2.5 \times 0.1211 \div 0.1 = 3.0275 \text{ mg/g mass}$.

Recent Product citations:

[1] Wang Y, Gao S, He X, et al. Response of total phenols, flavonoids, minerals, and amino acids of four edible fern species to four shading treatments[J]. PeerJ, 2020, 8: e8354.

Related Products:

AK0254/AK0253	Ceruloplasmin (CP) Assay Kit
AK0456/AK0455	Total antioxidant capacity (T-AOC) Assay Kit
AK0444/AK0443	Total Sulphydryl Assay Kit