

Plant Proanthocyanidins 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

Catalog Number: AK0447-100T-48S

Product Composition: Before use, please carefully check whether the volume of the reagent is consistent with the volume in the bottle.

Reagent name	Size	Preservation Condition
Extract Solution	Liquid 80 mL×1	2-8°C
Reagent I	Liquid 8 mL×1	2-8°C
Reagent II	Powder×1	2-8°C
Standard	Powder×1	2-8°C

Solution preparation:

- 1. Reagent II:** Dissolve with 8 mL of extract solution before use, the configured reagent II can be stored at 2-8°C for 1 month.
- 2. Standard:** 10 mg of Proanthocyanidins. Add 1mL of extraction solution before use, fully dissolve to obtain 10 mg/mL standard solution, and store at 2-8°C for two weeks;
- 3. Working Solution:** Mix Reagent I and II in a 1:1 ratio according to the dosage before use, and prepare them as needed. Mix as much as you need.

Product Description:

Oligomeric proantho cyanidins (OPC) is a polyphenol compound of a flavanol monomer and polymer, which exists widely in various organs of plants. It has strong oxidation resistance and the ability of scavenging free radical. It used widely in pharmaceutical, food, cosmetics, health care products and so on.

Under acidic conditions, resorcinol and pyrogallol in A ring of plant OPC can react with vanillin to form colored compound, which can be detected by colorimetric assay at 500 nm and calculate the content of OPC.

Technical Indicators:

Minimum detection limit: 0.0513 mg/mL

linear range: 0.078-5 mg/mL

Reagents and Equipment Required but Not Provided:

Visible spectrophotometer/microplate reader, micro glass cuvette/96 well plate, balance, centrifuge, crusher, ultrasonic cleaner, 30-50 mesh sieve and distilled water.

Operation procedure

I. Sample preparation.(The sample size to be tested can be adjusted appropriately, and the specific proportion can be referred to the literature.)

1. Dry the sample to constant mass, crush and filtrate with 30-50 mesh sieve, add 1 mL of extract solution to 0.1 g of sample, ultrasonic (power 300W) for 30 min, centrifuge at 12000 rpm and 25°C for 10 min. Add extract solution to supernatant, make final volume to 1 mL for test.

II. Determination procedure

1. Preheat spectrophotometer/microplate reader for 30 min, adjust the wavelength to 500 nm, The spectrophotometer needs to be zeroed with distilled water.

2. Preparation of standard solution: Dilute 10mg/mL standard solution with **Extract solution** to 4, 2.5, 1.25, 0.625, 0.3125, 0.15625 mg/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	10	200	300	4
2	10	125	375	2.5
3	2.5	500	500	1.25
4	1.25	500	500	0.625
5	0.625	500	500	0.3125
6	0.3125	500	500	0.15625

Note: Each standard tube in the following experiment requires 40 μL of standard solution (be careful not to directly test the absorbance of the standard solution in this step).

4. Add the following reagents:

Reagent Name	Blank Tube (A _B)	Standard Tube (A _S)	Test Tube (A _T)	Control Tube (A _C)
Sample (μL)	-	-	40	40
Standard (mg/mL)	-	40	-	-
Working Solution (μL)	160	160	160	-
H ₂ O (μL)	40	-	-	160

Mix thoroughly, 30°C water bath for 30 min, take 200 μL to micro cuvette/96 well plate, detect absorbance at 500 nm, $\Delta A(\text{Standard}) = \Delta A(S) = A_S - A_B$, $\Delta A(\text{Test}) = \Delta A(T) = A_T - A_C$. The standard curve and blank tube only need to be measured 1-2 times.

III. Calculation:

1. Make standard curve:

According to concentration of standard solution and absorbance to create the standard curve, take standard solution as X-axis, $\Delta A(T)$ as Y-axis. Take $\Delta A(S)$ into the equation to obtain x (mg/mL).

2. Calculation of OPC

The determination of ΔA is introduced into the equation and x(mg/mL) is obtained.

1. Calculated based on sample mass:

$$\text{OPC (mg/g mass)} = x \times V_e \div W = x \div W$$

2. Calculated based on sample protein concentration:

$$\text{OPC (mg/mg prot)} = x \times V_e \div (C_{pr} \times V_e) = x \div C_{pr}$$

C_{pr}: Sample protein concentration, mg/mL;

W: Sample mass. g;

V_e: Extraction volume, 1 mL;

Note:

If the measured absorbance value exceeds the linear range, the sample size can be increased or the sample can be diluted before measurement, and attention should be paid to synchronously modifying the calculation formula.

