Plant Flavonoids 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: BC1335

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 60 mL×1 (Required but not provided)	RT
Reagent I	Solution 2 mL×1	2-8℃
Reagent II	Solution 2 mL×1	2-8℃
Reagent III	Solution 15 mL×1	2-8℃
Standard	Powder×1	2-8℃
Standard diluent	Solution 15 mL×1	2-8℃

Solution preparation:

1. Extract: self-prepared 60% ethanol, stored at room temperature.
2. Standard: 10 mg rutin. Add 1 mL of standard diluent to prepare 10 mg/mL standard solution before use, the unused reagent can be stored at 2-8℃ for 4 weeks.

Product Description:

Flavonoids are a class of poly-phenyl compounds, which are plant secondary metabolites. They have the advantages of anti-inflammatory, antibacterial, hypolipemic, scavenging hydroxyl free radicals and cancer prevent.

In the alkaline nitrite solution, the flavonoid and the aluminum ion can form a red complex with a characteristic absorption peak at 470 nm. The sample flavonoid content can be calculated by measuring the absorbance of the sample extract at 470 nm.

Technical index:

Minimum detection limit: 0.00818 mg/mL

linear range: 0.0097-5 mg/mL

Reagents and Equipment Required but Not Provided:

Spectrophotometer/microplate reader, micro glass cuvette/ 96 well plate, balance, oven, crusher, 30-50 mesh sieve, ultrasonic cleaner, **60% ethanol,** adjustable pipette, centrifuge, mortar, distilled water, water bath/constant temperature incubator.

Sample Preparation:

The sample is dried to constant weight, pulverized, and after passing through a 30-50 mesh sieve, about 0.1 g is weighed, 1 mL of the Extract is added, and extraction is performed by ultrasonic extraction for 30min (ultrasonic power is 300 W, 60℃, total time 30 min). Centrifuge at 12000 rpm and 25℃ for 10 min, take the supernatant, and dilute to 1 mL with the extract.

Procedure:

1. Preheat spectrophotometer/microplate reader for 30min, adjust the wavelength to 470 nm. The spectrophotometer needs to be zeroed with distilled water.
2. Preparation of standard solution: Dilute 10 mg/mL rutin standard solution with standard diluent for 2.5, 1.25, 0.625, 0.3125, 0.15625, 0.078, 0.039 mg/mL for use.

Number	Pre dilution concentration (mg/mL)	Standard liquid volume (μL)	Volume of standard dilution solution (μL)	Diluted concentration (mg/mL)
1	10	250	750	2.5
2	2.5	200	200	1.25
3	1.25	200	200	0.625
4	0.625	200	200	0.3125
5	0.3125	200	200	0.15625
6	0.15625	200	200	0.078
7	0.078	200	200	0.039
8	0.039	200	200	0.02

Note: Each standard tube in the following experiment requires 60 μL of standard solution (be careful not to directly test the absorbance of the standard solution in this step).

3. Operation table

Reagent Name (μL)	Control Tube (Ac)	Test Tube (At)	Standard Tube (As)	Blank Tube (Ab)
Sample	60	60	-	-
Standard	-	-	60	-
Distilled H_2O	-	-	-	60
Reagent I	15	15	15	15
Mix and react for 5 min at room temperature				
Reagent II	-	15	15	15
Mix and react for 5 min at room temperature				
Reagent III	120	120	120	120
60% ethanol	105	90	90	90

Mix thoroughly, react for 45 min at 37℃ water bath/constant temperature incubator, then centrifuge at 10000 g for 10 min, take 200 μL into micro glass cuvette/ 96 well plate and detect absorbance at 470nm, name Ac, At, As, Ab. Calculate ΔA(standard) =ΔA(S) =As-Ab, ΔA(test)

=ΔA(T) =At-Ac. Each Test tube

needs to be provided with a control tube. The standard curve and blank tube only need to be measured 1-2 times.

Calculation:

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

2.Calculated according to the weight of the sample:

flavonoid content (mg/g weight) = x × V_E ÷ W=x ÷ W

3.Calculated according to the sample protein concentration:

flavonoid content (mg/mg prot) = x × V_E ÷ (Cpr × V_E) = x ÷ Cpr

V_E: volume of added extraction solution, 1 mL;

W: weight of sample, g;

Cpr: concentration of sample protein, mg/mL.

Note:

1. If the absorbance value exceeds the linear range, the sample size can be increased or diluted before proceeding with the measurement. Pay attention to synchronously modifying the calculation formula.
2. After color development is completed, detect the sample absorbance immediately. The absorbance will decrease after 2 hours.

Examples:

1. Add 0.1g treated grape peel to 1mL extract solution, follow the determination procedure to operate, and calculate: ΔA =At-Ac=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 (μmol/g mass)=x÷W=0.789÷0.1=7.89 mg/g weight.

Recent Product citations:

[1] Han Q, Song H, Yang C, Zhang S, Korpelainen H, Li C. Integrated DNA methylation, transcriptome and physiological analyses reveal new insights into superiority of poplars formed by interspecific grafting. Tree Physiol. 2022 Jul 5;42(7):1481-1500. doi: 10.1093/treephys/tpac013. PMID: 35134240.

[2] 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.

[3] Liao X, Rao S, Yu T, Zhu Z, Yang X, Xue H, Gou Y, Cheng S, Xu F. Selenium yeast

promoted the Se accumulation, nutrient quality and antioxidant system of cabbage (Brassica oleracea var. capitata L.). Plant Signal Behav. 2021 Jun 3;16(6):1907042. doi: 10.1080/15592324.2021.1907042. Epub 2021 Apr 5. PMID: 33818289; PMCID: PMC8143226.

[4] Hou Q, Li S, Shang C, Wen Z, Cai X, Hong Y, Qiao G. Genome-wide characterization of chalcone synthase genes in sweet cherry and functional characterization of CpCHS1 under drought stress. Front Plant Sci. 2022 Aug 19;13:989959. doi: 10.3389/fpls.2022.989959. PMID: 36061761; PMCID: PMC9437463.

[5] Cai N, Nong X, Liu R, McNeill MR, Wang G, Zhang Z, Tu X. The Conserved Cysteine-Rich Secretory Protein MaCFEM85 Interacts with MsWAK16 to Activate Plant Defenses. Int J Mol Sci. 2023 Feb 17;24(4):4037. doi: 10.3390/ijms24044037. PMID: 36835451; PMCID: PMC9967070.

Reference:

[1] Nabavi S, Ebrahimzadeh M, Nabavi S. Determination of antioxidant activity, phenol and flavonoid content of Parrotia persica Mey [J]. Pharmacologyonline, 2008, 2: 560-567.

Related Products:

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