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

Catalog Number: BC1330

Size:50T/24S

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 50 mL×1 (Required but not provided)	RT
Reagent I	Solution 5 mL×1	2-8℃
Reagent II	Solution 4 mL×1	2-8℃
Reagent III	Solution 30 mL×1	2-8℃
Standard	Powder×1	2-8℃
Standard diluent	Solution 20 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: 0.00666 mg/mL

linear range: 0.0097-1.75 mg/mL

Reagents and Equipment Required but Not Provided:

Spectrophotometer, 1mL glass cuvette, balance, oven, comminution apparatus, 30-50 mesh sieve, ultrasonic cleaner, 60% ethanol, 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 for 30 min, adjust the wavelength to 470 nm and set the counter to zero with distilled water.
2. Dilute 10 mg/mL rutin standard solution with standard diluent for 1.5, 1.25, 0.625, 0.3125, 0.156, 0.078, 0.039, 0.02 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	150	850	1.5
2	10	125	875	1.25
3	1.25	500	500	0.625
4	0.625	500	500	0.3125
5	0.3125	500	500	0.15625
6	0.15625	500	500	0.078
7	0.078	500	500	0.039
8	0.039	500	500	0.02

Note: Each standard tube in the following experiment requires 200 μL of standard solution (be careful not to directly test the absorbance of the standard solution in this step).

3. Operation table

Reagent Name (mL)	Control Tube (Ac)	Test Tube (At)	Standard Tube (As)	Blank Tube (Ab)
Sample	0.2	0.2	-	-
Standard	-	-	0.2	-
Distilled water	-	-	-	0.2
Reagent I	0.05	0.05	0.05	0.05
Mix and react for 5 min at room temperature				
Reagent II	-	0.05	0.05	0.05
Mix and react for 5 min at room temperature				
Reagent III	0.4	0.4	0.4	0.4
60% ethanol	0.35	0.3	0.3	0.3

Mix thoroughly, react for 45 min at 37℃ water bath/constant temperature incubator, then centrifuge at 10000×g for 10 min, take supernatant in 1 mL glass cuvette and measure absorbance at

470 nm, 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 fresh weight of the sample:
   flavonoid content (mg/g weight) = $x \times V_E \div W = x \div W$
3. Calculated according to sample protein concentration:
   flavonoid content (mg/mg prot) = $x \times V_E \div (Cpr \times V_E) = x \div Cpr$
   V_E: volume of added extraction solution, 1 mL;
   W: fresh 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, use ultrasonic wave to crack, take supernatant and follow the determination procedure to operate, and calculate: ΔA =At-Ac=0.675-0.325=0.350, standard curve: y=0.6197x-0.0059, calculate x=0.5743, according with weight of sample to calculate: Flavonoid content (μmol/g mass) = x÷W=0.5743÷0.1=5.743 mg/g mass.

Recent Product citations:

[1] Gong Z, Duan Y, Liu D, Zong Y, Zhang D, Shi X, Hao X, Li P. Physiological and transcriptome analysis of response of soybean (Glycine max) to cadmium stress under elevated CO2 concentration. J Hazard Mater. 2023 Apr 15;448:130950. doi: 10.1016/j.jhazmat.2023.130950. Epub 2023 Feb 10. PMID: 36860078.

[2] Zeeshan M, Hu YX, Guo XH, Sun CY, Salam A, Ahmad S, Muhammad I, Nasar J, Jahan MS, Fahad S, Zhou XB. Physiological and transcriptomic study reveal SeNPs-mediated AsIII stress detoxification mechanisms involved modulation of antioxidants, metal transporters, and

transcription factors in Glycine max L. (Merr.) roots. Environ Pollut. 2023 Jan 15;317:120637. doi: 10.1016/j.envpol.2022.120637. Epub 2022 Nov 16. PMID: 36400144.

[3] Li X, Cao L, Jiao B, Yang H, Ma C, Liang Y. The bHLH transcription factor AcB2 regulates anthocyanin biosynthesis in onion (Allium cepa L.). Hortic Res. 2022 Jun 2;9:uhac128. doi: 10.1093/hr/uhac128. PMID: 36042846; PMCID: PMC9418810.

[4] Wang Y, Zhang Y, Yuan Y, Zhao Y, Nie J, Nan T, Huang L, Yang J. Nutrient content prediction and geographical origin identification of red raspberry fruits by combining hyperspectral imaging with chemometrics. Front Nutr. 2022 Oct 17;9:980095. doi: 10.3389/fnut.2022.980095. PMID: 36386936; PMCID: PMC9642070.

[5] Lu X, Chen G, Ma L, Zhang C, Yan H, Bao J, Nai G, Wang W, Chen B, Ma S, Li S. Integrated transcriptome and metabolome analysis reveals antioxidant machinery in grapevine exposed to salt and alkali stress. Physiol Plant. 2023 May-Jun;175(3):e13950. doi: 10.1111/ppl.13950. PMID: 37291799.

Reference:

[1] Takeshi Nagai, Reiji Inoue, Hachiro Inoue. et al. Scavenging capacities of pollen extracts from cistus ladaniferus on autoxidation, superoxide radicals, hydroxyl radicals, and DPPH radicals [J]. Nutrition Research, 2002, 22(4): 519-526.

[2] Tsai CH, Stern A, Chiou JF. et al. Rapid and specific detection of hydroxyl radical using an ultraweak chemiluminescence analyzer and a low-level chemiluminescence emitter: application to hydroxyl radical-scavenging ability of aqueous extracts of Food constituents[J]. Journal of Agricultural and Food Chemistry, 2001, 49(5): 2137-2141.

Related Products:

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