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Plant flavonoids Assay Kit

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

Operation Equipment: Spectrophotometer/ microplate reader

Catalog Number: AK0451

Size:100T/48S

Product composition:

Extract : Self-prepared, stored at room temperature

Reagent I: Liquid 2 mL×1, Storage at 4℃

Reagent II: Liquid 2 mL×1, Storage at 4℃

Reagent III: Liquid 15 mL×1, Storage at 4℃

Standard: Powder×1, 10 mg of rutin standard solution, Storage at 4℃. Add 1 mL of standard diluent to prepare 10 mg/mL standard solution before use

Standard diluent: Liquid 15 mL×1, stored at 4℃.

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.

Reagents and Equipment Required but Not Provided:

Spectrophotometer/ microplate reader, adjustable pipette, balance, oven, sieve, comminution apparatus, sonic breaker, centrifuge, micro glass cuvette/ 96 well flat-bottom plate, 60% ethanol and distilled water.

Sample preparation:

The sample is dried to constant weight, pulverized, and after passing through a 40 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, crushed for 5 s, intermittently 8 s, 60℃, total time 30min). Centrifuge at 12000 rpm and 25℃ for 10 min, take the supernatant, and dilute to 1 mL with the extract.

Procedure:

1. The 10 mg/mL rutin standard solution is dilute to 5, 2.5, 1.25, 0.625, 0.3125, 0. 156, 0.078, 0.039 mg/mL for use.

2. Preheat spectrophotometer/microplate reader for 30min, adjust the wavelength to 470 nm and set the counter to zero with distilled water.

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	105	105	105

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

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 fresh weight) = $x \times V_E \div W = x \div W$

3.Calculated according to the sample protein concentration:

flavonoid content (mg / mg prot) = $x \times V_E \div (Cpr \times VE) = x \div Cpr$

V_E : volume of added extraction solution, 1 mL;

W: fresh weight of sample, g;

Cpr: concentration of sample protein, mg/mL.

Note:

1. Dilute sample with extract solution if OD> 1. Note that the calculation formula is multiplied by the dilution factor.

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, with 300w at 60 C , break for 5 s and interrupt for 8s, 30 min for whole process, centrifuge with 12000 rpm at 25 C for 1 0min, take supernatant and add extract solution to 1 ml, follow the determination procedure to operate, and calculate: ΔA =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 （ μmol/g mass ） =x÷W=0.789÷0. 1=7.89 mg/g mass.

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