# **Plant Flavonoids Assay Kit**

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

**Operation Equipment:** Spectrophotometer

Catalog Number: BC1330

**Size**:50T/24S

## **Product composition:**

Extract: self-prepared, stored at room temperature.

Reagent II: Liquid 5 mL×1 Storage at 4°C. Reagent III: Liquid 4 mL×1 Storage at 4°C. Reagent III: Liquid 30mL×1 Storage at 4°C.

Standard: Liquid 1 mL×1, 10 mg of rutin standard solution, Storage at 4°C.

Standard diluent: Liquid 20 mL×1, stored at 4°C.

## **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, balance, oven, sieve, comminution apparatus, sonic breaker, centrifuge, 1mL glass cuvette, 60% ethanol, 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°C, total time 30 min). Centrifuge at 12000 rpm and 25°C for 10 min, take the supernatant, and dilute to 1 mL with the extract.

#### **Procedure:**

- 1. The 10 mg/mL rutin standard solution, dilute to 1.5, 1.25, 0.625, 0.3125, 0.156, 0.078, 0.039, 0.02 mg/mL for use.
- 2. Preheat spectrophotometer for 30 min, adjust the wavelength to 470 nm and set the counter to zero with distilled water.
- 3. Add reagent to a 1.5mL EP tube:

ĺ	Reagent name (mL)	Control tube (Ac)	Test tube (At)	Standard tube (As)	Blank tube (Ab)

Sample	0.2	0.2	-	-		
Standard	-	-	0.2	-		
Distilled H <sub>2</sub> O	-	-	-	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	0.35	0.35	0.35		

Mix thoroughly, react for 45 min at 37°C water bath, then centrifuge at 10000g for 10min. set the counter to zero with control tube. measure absorbance at 470 nm, name Ac, At, As, Ab. calculate  $\Delta A(\text{standard}) = \Delta A(S) = As - Ab$ ,  $\Delta A(\text{test}) = \Delta A(T) = At - Ac$ .

#### **Calculation:**

- 1.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. 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) =  $x \times V_E \div (Cpr \times V_E) = x \div Cpr$ 

V<sub>E</sub>: 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  $^{\circ}$ C, break for 5s and interrupt for 8s, 30min for whole process, centrifuge with 12000rpm at 25  $^{\circ}$ C for 10min, take supernatant and add extract solution to 1ml, follow the determination procedure to operate, and calculate:  $\Delta A = A(T)-A(B)=0.675-0.325=0.350$ , standard curve: y=0.6197x-0.0059, calculate x=0.5743, according with mass of sample to calculate: Flavonoid content (  $\mu$ mol/g mass)  $=x\div W=0.5743\div 0.1=5.743$  mg/g mass.

#### **Related Products:**

BC1300/BC1305 Ceruloplasmin (CP) Assay Kit

BC1310/BC1315 Total antioxidant capacity (T-AOC) Assay Kit

BC1370/BC1375 Total Sulfhydryl Assay Kit