

## Plant Flavonoids Assay Kit

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

**Operation Equipment:** Spectrophotometer

**Catalog Number:** AK0452

**Size:**50T/24S

### Product composition:

Extract: self-prepared, stored at room temperature.

Reagent I: Liquid 5 mL×1 Storage at 4°C .

Reagent II: 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, 1 mL 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:

Reagent name (mL)	Control tube (Ac)	Test tube (At)	Standard tube (As)	Blank tube (Ab)
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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  $\Delta A(\text{standard}) = \Delta A(S) = A_s - A_b$ ,  $\Delta A(\text{test}) = \Delta A(T) = A_t - A_c$ .

#### 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:

$$\text{flavonoid content (mg/g fresh weight)} = x \times V_E \div W = x \div W$$

3. Calculated according to The sample protein concentration:

$$\text{flavonoid content (mg / mg prot)} = x \times V_E \div (C_{pr} \times V_E) = x \div C_{pr}$$

$V_E$ : volume of added extraction solution, 1 mL;

W: fresh weight of sample, g;

$C_{pr}$ : 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 5s and interrupt for 8s, 30min for whole process, centrifuge with 12000rpm at 25C 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 (  $\mu\text{mol/g mass}$  )  $= x \div W = 0.5743 \div 0.1 = 5.743 \text{ mg/g mass}$ .



**Related Products:**

AK0456/AK0455	Total antioxidant capacity (T-AOC) Assay Kit
AK0444/AK0443	Total Sulphydryl Assay Kit
AK0254/AK0253	Ceruloplasmin (CP) Assay Kit