

## EZMeta™ Plant Flavonoids Colorimetric Assay Kit

Cat #: D-AKC1530

Size: 48T / 96T

Storage: Stored at 4°C for 6 months, protected from light

### Product Information

**Detection range:** 0.156-10 mg/g

**Sensitivity:** 0.078 mg/g

**Applicable samples:** Plant Tissues

### Assay Principle

Flavonoids are a class of polyphenyl compounds, which are secondary metabolites of plants. They have anti-inflammatory, antibacterial, hypolipidemic effects on the human body, scavenging hydroxyl free radicals in the body and preventing cancer. Quercetin is a typical flavonoid. CheKine™ Micro Plant Flavonoids Assay Kit provides a simple method for detecting Flavonoids concentration in a variety of Plant Tissues. In the assay, in the alkaline nitrite solution, the flavonoids and aluminum ions in the plant samples form a red complex with a characteristic absorption peak at 502 nm. The flavonoid content of the sample can be calculated by measuring the absorbance of the sample extract at 502 nm. The kit can detect plant samples.

## Materials Supplied and Storage Conditions

| Kit components                | Size   |        | Storage conditions        |
|-------------------------------|--------|--------|---------------------------|
|                               | 48 T   | 96 T   |                           |
| Nitrite Solution              | 1 mL   | 2 mL   | 4°C                       |
| Chromogen                     | 1 mL   | 2 mL   | 4°C                       |
| NaOH Solution                 | 7.5 mL | 15 mL  | 4°C                       |
| Quercetin Standard (10 mg/mL) | 0.1 mL | 0.2 mL | 4°C, protected from light |

## Materials Required but Not Supplied

- Microplate reader or visible spectrophotometer capable of measuring absorbance at 502 nm
- 96-well plate or microglass cuvette, precision pipettes, disposable pipette tips
- Incubator, refrigerated centrifuge, ultrasonic disruptor, water bath
- Deionized water, 60% ethanol
- Pulverizer (or wall breaker), 40 mesh screen

## Reagent Preparation

**Extraction Buffer (Please prepare yourself):** 60% ethanol; Store at room temperature.

**Nitrite Solution:** Ready to use as supplied. keep balance with room temperature before starting the assay; Store at 4°C.

**Chromogen:** Ready to use as supplied. keep balance with room temperature before starting the assay; Store at 4°C.

**NaOH Solution:** Ready to use as supplied. keep balance with room temperature before starting the assay; Store at 4°C.

**Standard curve setting:** Dilute 50 µL 10 mg/mL Quercetin Standard to 1 mg/mL with 450 µL 60% ethanol. And further dilute the standard to 1, 0.5, 0.25, 0.125, 0.0625, 0.0313, 0.0156 mg/mL standard solution with 60% ethanol, as shown in the following table.

| Num.  | Volume of Standard                  | Volume of 60% Ethanol<br>( $\mu$ L) | The Concentration of Standard<br>(mg/mL) |
|-------|-------------------------------------|-------------------------------------|------------------------------------------|
| Std.1 | 50 $\mu$ L 10 mg/mL                 | 450                                 | 1                                        |
| Std.2 | 100 $\mu$ L of Std.1 (1 mg/mL)      | 100                                 | 0.5                                      |
| Std.3 | 100 $\mu$ L of Std.2 (0.5 mg/mL)    | 100                                 | 0.25                                     |
| Std.4 | 100 $\mu$ L of Std.3 (0.25 mg/mL)   | 100                                 | 0.125                                    |
| Std.5 | 100 $\mu$ L of Std.4 (0.125 mg/mL)  | 100                                 | 0.0625                                   |
| Std.6 | 100 $\mu$ L of Std.5 (0.0625 mg/mL) | 100                                 | 0.0313                                   |
| Std.7 | 100 $\mu$ L of Std.6 (0.0313 mg/mL) | 100                                 | 0.0156                                   |

**Note:** Always prepare fresh standards per use; Diluted standard solution is unstable and should not be stored for a long time.

## Sample Preparation

1. For plant tissues with more fibers, the plant samples can be dried to constant weight, pulverized and sieved by a 40-mesh sieve, weigh about 0.1 g, add 1 mL of Extraction Buffer, and extracted by ultrasonic extraction ( power 300 w, ultrasound 5 s, 8s gap, total time 30 min, and temperature 60°C). Centrifuge at 12,000 rpm for 10 min at 25°C, take the supernatant, and dilute the volume to 1 mL with Extraction Buffer for further test.

2. For delicate plants tissues with less fiber, you can directly weigh about 0.1 g of fresh tissue, add 1 mL Extraction Buffer, homogenized with homogenizer, and extracted by ultrasonic extraction (power 300w, ultrasound 5 s, 8s gap, total time 30 min, and temperature 60°C). Centrifuge at 12,000 rpm for 10 min at 25°C, take the supernatant, and dilute the volume to 1 mL with Extraction Buffer for further test.

**Note:** It will be better to quantify the total protein with EZMeta™ Plant Flavonoids Colorimetric Assay Kit, if the content is calculated by protein concentration.

## Assay Procedure

1. Preheat the microplate reader or visible spectrophotometer for more than 30 min, and adjust the wavelength to 502 nm, visible spectrophotometer was returned to zero with deionized water.

2. Sample measurement (the following operations are operated in the EP tube):

| Reagent                                              | Blank Tube(μL) | Standard Tube(μL) | Test Tube(μL) | Control Tube(μL) |
|------------------------------------------------------|----------------|-------------------|---------------|------------------|
| Sample                                               | 0              | 0                 | 60            | 60               |
| Different Concentration of Std.                      | 0              | 60                | 0             | 0                |
| Deionized Water                                      | 60             | 0                 | 0             | 0                |
| Nitrite Solution                                     | 15             | 15                | 15            | 15               |
| Mix well and incubate at room temperature for 5 min. |                |                   |               |                  |
| Chromogen                                            | 15             | 15                | 15            | 0                |
| Mix well and incubate at room temperature for 5 min. |                |                   |               |                  |
| NaOH Solution                                        | 120            | 120               | 120           | 120              |
| 60% ethanol                                          | 90             | 90                | 90            | 105              |

Mix well and incubate at room temperature for 15 min, transfer 200 μL to 96-well plate or microglass cuvette. Then

reading the values at 502 nm, marked as  $A_{\text{Blank}}$ ,  $A_{\text{Standard}}$ ,  $A_{\text{Test}}$  and  $A_{\text{Control}}$ . Finally, calculate  $\Delta A_{\text{Test}} = A_{\text{Test}} - A_{\text{Control}}$ ;

$\Delta A_{\text{Standard}} = A_{\text{Standard}} - A_{\text{Blank}}$ . Blank tube only needs to measure 1 time.

**Note:** In order to guarantee the accuracy of experimental results, need to do a pre-experiment with 2-3 samples. Each sample needs to be set with a control well. If the  $\Delta A_{\text{Test}}$  is greater than 0.6, the sample needs to be properly diluted with 60% ethanol before measurement. Pay attention to the calculation formula to multiply by the dilution factor. Measure immediately after the color development is completed, and the absorbance value will decrease after 2 h.

## Data Analysis

**Note:** We provide you with calculation formulae, including the derivation process and final formula. The two are exactly equal. It is suggested that the concise calculation formula in bold is final formula.

1. Drawing of standard curve

With the concentration of the standard solution as the y-axis and the  $\Delta A_{\text{Standard}}$  as the x-axis, draw the standard curve.

## 2. Calculating the concentration of flavonoid

Bring the  $\Delta A_{\text{Test}}$  of the sample into the equation to get the y value (mg/mL).

(1) Calculated by weight of samples

$$\text{Flavonoid content (mg/g dry weight or fresh weight)} = y \times V_{\text{Extraction}} \div W \times n = 10y \times n$$

(2) Calculated by protein concentration

$$\text{Flavonoid content (mg/mg prot)} = y \times V_{\text{Extraction}} \div (\text{Cpr} \times V_{\text{Extraction}}) \times n = y \div \text{Cpr} \times n$$

Where:  $V_{\text{Extraction}}$ : Extraction buffer added, 1 mL; W: the weight of samples, 0.1 g; n: dilution multiple of sample further dilution;

Cpr: sample protein concentration, mg/mL.

## Typical Data

Typical standard curve:

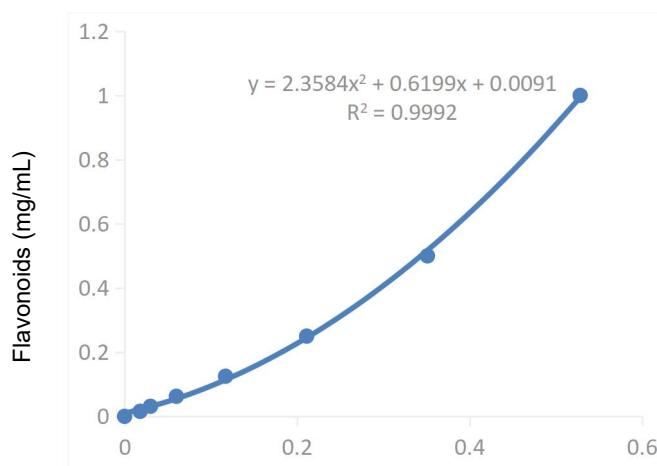


Figure 1. Standard curve for Flavonoids.

## Disclaimer

The reagent is only used in the field of scientific research, not suitable for clinical diagnosis or other purposes.