# CheKine™ Plant Flavonoids Colorimetric Assay Kit

Cat #: KTB1530 Size: 48 T/96 T

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|---|---|---|---|
| | **Plant Flavonoids Colorimetric Assay Kit** | | |
| REF | **Cat #:** KTB1530 | LOT | **Lot #:** Refer to product label |
| | **Detection range:** 0.156-10 mg/g | | **Sensitivity:** 0.078 mg/g |
| | **Applicable samples:** Plant Tissues | | |
| | **Storage:** Stored at 4℃ for 6 months | | |

## 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™ Plant Flavonoids Colorimetric 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℃ |
| Chromogen | 1 mL | 2 mL | 4℃ |
| NaOH Solution | 7.5 mL | 15 mL | 4℃ |
| Quercetin Standard (10 mg/mL) | 0.1 mL | 0.2 mL | 4℃, protected from light |

## Materials Required but Not Supplied

- Microplate Reader capable of measuring absorbance at 502 nm
- Incubator, Refrigerated Centrifuge and Ultrasonic disruptor
- 96 well plate with clear flat bottom, Precision Pipettes, Disposable Pipette Tips
- Deionized Water and 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℃.

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

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

**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.

| | Volume of Standard | Volume of 60% ethanol (μL) | The concentration of Standard（mg/mL） |
|---|---|---|---|
| Std.1 | 50 μL 10 mg/mL | 450 | 1 |
| Std.2 | 100 μL of Std.1 (1 mg/mL) | 100 | 0.5 |
| Std.3 | 100 μL of Std.2 (0.5 mg/mL) | 100 | 0.25 |
| Std.4 | 100 μL of Std.3 (0.25 mg/mL) | 100 | 0.125 |
| Std.5 | 100 μL of Std.4 (0.125 mg/mL) | 100 | 0.0625 |
| Std.6 | 100 μL of Std.5 (0.0625 mg/mL) | 100 | 0.0313 |
| Std.7 | 100 μL 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℃). Centrifuge at 12,000 rpm for 10 min at 25℃, 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℃). Centrifuge at 12,000 rpm for 10 min at 25℃, take the supernatant, and dilute the volume to 1 mL with Extraction Buffer for further test.

## Assay Procedure

1. Preheat the microplate reader for more than 30 min, and adjust the wavelength to 502 nm.

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

| | 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. Then reading the values at 502 nm, marked as $A_{Blank}$, $A_{Standard}$, $A_{Test}$ and $A_{Control}$. Finally, calculate $\Delta A_{Test} = A_{Test} - A_{Control}$; $\Delta A_{Standard} = A_{Standard} - A_{Blank}$. Blank Tube only need 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 $\Delta A_{Test}$ 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 hours. If you need to determine the protein concentration of the sample, it is recommended to use Abbkine Cat #: KTD3001 Protein Quantification Kit (BCA Assay) to measure the protein concentration of the sample.**

## 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 $\Delta A_{Standard}$ as the x-axis, draw the standard curve.

2. Calculating the concentration of flavonoid:

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

(1) Calculated by weight of samples

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

(2) Calculated by protein concentration

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

Where: $V_{Extraction}$: 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

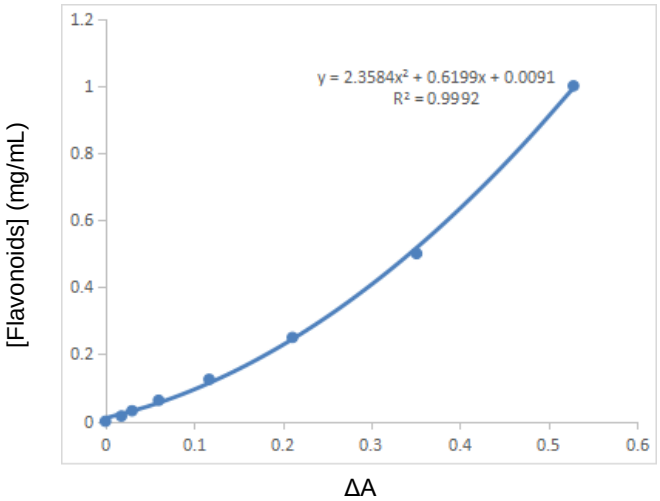


Figure1. Standard Curve of Flavonoids in 96-well plate assay–data provided for demonstration purposes only. A new standard

Curve must be generated for each assay.

## Recommended Products

| Catalog No. | Product Name |
|---|---|
| KTB1500 | CheKine™ Total Antioxidant Capacity (TAC) Assay Kit (Colorimetric) |
| KTB1080 | CheKine™ Superoxide anion Scavenging Capacity Assay Kit (Colorimetric) |
| KTB1090 | CheKine™ Hydroxyl Free Radical Scavenging Capacity Assay Kit (Colorimetric) |
| KTB1510 | CheKine™ Uric Acid (UA) Colorimetric Assay Kit |
| KTB1520 | CheKine™ Plant Oligomeric Proantho Cyanidins (OPC) Colorimetric Assay Kit |
| KTB1540 | CheKine™ Plant Total Phenols (TP) Colorimetric Assay Kit |

## Disclaimer

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