CheKine™ Micro Plant Flavonoids Assay Kit

Cat #: KTB1530 Size: 48 T/96 T

	Micro Plant Flavonoids 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, protected from light		

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℃
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 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℃.

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.

Num.	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.

Note: It will be better to quantify the total protein with Protein Quantification Kit (BCA Assay), Cat #: KTD3001, 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_{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 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 ΔA_{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 $\Delta A_{Standard}$ as the x-axis, draw the standard curve.

2. Calculating the concentration of flavonoid

Bring the ΔA_{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×n**

(2) Calculated by protein concentration

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

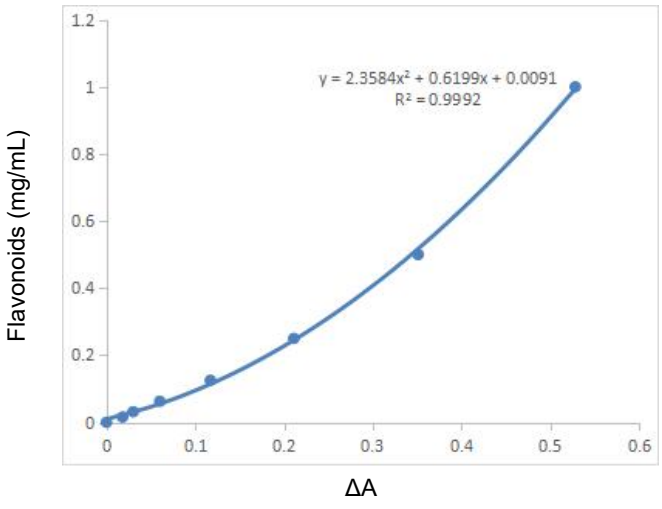

Figure 1. Standard curve for Flavonoids.

Recommended Products

Catalog No.	Product Name
KTB1500	CheKine™ Micro Total Antioxidant Capacity (TAC) Assay Kit
KTB1080	CheKine™ Micro Superoxide anion Scavenging Capacity Assay Kit
KTB1091	CheKine™ Micro Hydroxyl Free Radical Scavenging Capacity Assay Kit
KTB1510	CheKine™ Micro Uric Acid (UA) Assay Kit
KTB1520	CheKine™ Micro Plant Oligomeric Proantho Cyanidins (OPC) Assay Kit
KTB1540	CheKine™ Micro Plant Total Phenols (TP) Assay Kit

Disclaimer

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