CheKine™ Micro Plant Total Phenols (TP) Assay Kit

Cat #: KTB1540 Size: 48 T/48 S 96 T/96 S

	Micro Plant Total Phenols (TP) Assay Kit		
REF	**Cat #:** KTB1540	LOT	**Lot #:** Refer to product label
	Detection range: 0.0078-0.5 mg/mL (0.195-12.5 mg/g)		**Sensitivity:** 0.0078 mg/mL (0.195 mg/g)
	Applicable samples: Plant Tissues and Liquid samples such as juice and honey		
	Storage: Stored at 4℃ for 6 months, protected from light		

Assay Principle

Plant phenols have the effects of scavenging free radicals, anti-oxidation and anti-aging, and have high nutritional value and medical and health care effects. They are widely used in cosmetics, food, medicine and other fields. CheKine™ Micro Total Phenols (TP) Assay Kit provides a convenient tool for detection of total phenols. The principle is that under alkaline conditions, phenolic substances reduce tungsten molybdenum acid to produce a blue compound with a characteristic absorption peak at 760 nm. The total phenol content of the sample can be obtained by measuring the absorbance at 760 nm.

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	48 T	96 T	
Assay Buffer	6.5 mL	13 mL	4℃
Chromogen	3 mL	6 mL	4℃, protected from light
Tannin Standard	Powder×1 vial	Powder×2 vials	4℃, protected from light

Note: Before formal testing, it is recommended to select 2-3 samples with large expected differences for pre-experiment.

Materials Required but Not Supplied

- Microplate reader or visible spectrophotometer capable of measuring absorbance at 760 nm
- 96-well plate or microglass cuvette, precision pipettes, disposable pipette tips
- Centrifuge, ultrasonic disruptor, incubator, water bath
- Deionized water, 60% ethanol
- Pulverizer (or wall breaker), 40 mesh screen

Reagent Preparation

Assay Buffer: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃.

Chromogen: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃, protected from light.

Note: Chromogen has certain irritation, so personal protection is recommended during use.

Tannin Standard: Add 1 mL of deionized water to dissolve before use. The concentration is 5 mg/mL, which could be stored at 4℃, protected from light for 1 week.

Standard curve setting: Further dilute the Standard to 0.5, 0.25, 0.125, 0.0625, 0.0313, 0.0156, 0.0078 mg/mL standard with deionized water, as shown in the following table.

Num.	Volume of Standard	Volume of Deionized Water (μL)	The Concentration of Standard (mg/mL)
Std.1	100 μL of 5 mg/mL	900	0.5
Std.2	100 μL of Std.1 (0.5 mg/mL)	100	0.25
Std.3	100 μL of Std.2 (0.25 mg/mL)	100	0.125
Std.4	100 μL of Std.3 (0.125 mg/mL)	100	0.0625
Std.5	100 μL of Std.4 (0.0625 mg/mL)	100	0.0313
Std.6	100 μL of Std.5 (0.0313 mg/mL)	100	0.0156
Std.7	100 μL of Std.6 (0.0156 mg/mL)	100	0.0078

Note: Always prepare fresh standards per use; Diluted Standard Solution is unstable and must be used within 4 h.

Sample Preparation

Note: Fresh samples are recommended. If not assayed immediately, samples can be stored at -80℃ for one month.

1. Plant tissue: After the samples were dried to a constant weight, pulverized and sieved by a 40-mesh sieve, weigh about 0.1 g, add 2.5 mL of 60% ethanol, and extracted by ultrasonic extraction (power 300 W, ultrasound 5 s, 8 s gap, total time 30 min, and 60℃). Centrifuge at 12,000 rpm for 10 min at 25℃, take the supernatant, and dilute the volume to 2.5 mL with the 60% ethanol for further test.
2. Liquid samples such as juice and honey: Tested directly.

Note: The extraction buffer contains components that denature the protein. If the protein concentration is calculated, the protein needs to be extracted with deionized water for determination. 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 760 nm, visible spectrophotometer was returned to zero with deionized water.
2. Sample measurement (add the following reagents in sequence into the 96-well plate or microglass cuvette).

Reagent	Blank Well (μL)	Standard Well (μL)	Test Well (μL)	Control Well (μL)
Sample	0	0	10	10
Different Concentration Std.	0	10	0	0
Deionized Water	10	0	0	0
Chromogen	50	50	50	0
Mix well and kept at room temperature for 2 min				
Assay Buffer	50	50	50	50
Deionized Water	90	90	90	140
Mix well and kept at room temperature for 10 min. Then reading the values at 760 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. If ΔA_{Test} is less than 0.004, increase the sample quantity appropriately. If ΔA_{Test} is greater than 1.0, the sample can be appropriately diluted with 60% ethanol, the calculated result multiplied by the dilution factor.

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 content of total phenols

Bring the ΔA_{Test} of the sample into the equation to get the y value (mg/mL).

(1) Calculated by weight of samples

total phenols content (mg/g weight)=$y \times V_{Sample} \div (W \times V_{Sample} \div V_{Extraction}) \times n = y \times V_{Extraction} \div W \times n$**$=2.5y \div W \times n$**

(2) Calculated by protein concentration

total phenols (mg/mg prot)=$y \times V_{Sample} \div (V_{Sample} \times Cpr) \times n$**$=y \div Cpr \times n$**

(3) Calculated by volume of Liquid samples

total phenols (mg/mL)=$y \times V_{Sample} \div V_{Sample} \times n$**$=y \times n$**

Where: V_{Sample}: add sample volume, 0.01 mL; W: weight of sample, g; $V_{Extraction}$: added Extraction Buffer volume, 2.5 mL; n: the sample dilution factor; Cpr: sample protein concentration, mg/mL.

Note: If the unit of mg/mL is needed to convert to mg/g, please divide it by the density of the liquid.

Typical Data

Typical standard curve

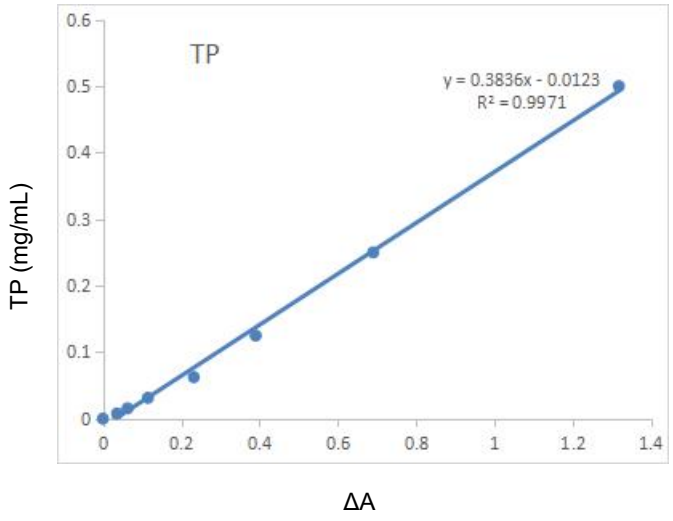

Figure1. Standard curve for total phenol.

Examples:

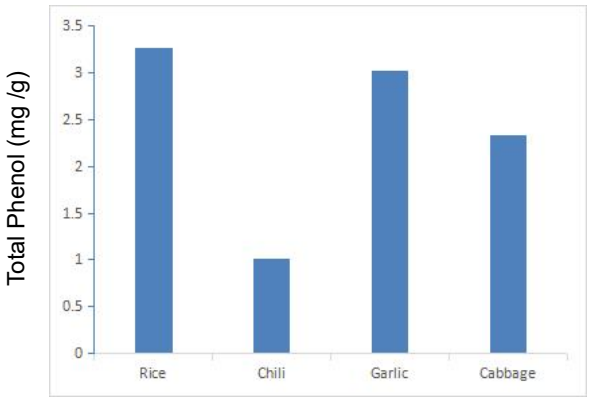

Figure 2. Total phenol concentration in rice, chili, garlic, and cabbage respectively. Assays were performed following kit protocol.

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
KTB1530	CheKine™ Micro Plant Flavonoids Assay Kit

Disclaimer

The reagent is only use in the field of scientific research, not suitable for clinical diagnosis or other purposes. For your safety and health, please wear a lab coat and disposable gloves.