CheKine™ Tannin Colorimetric Assay Kit

Cat #: KTB1541 Size: 48 T/96 T

	Tannin Colorimetric Assay Kit		
REF	**Cat #:** KTB1541	LOT	**Lot #:** Refer to product label
	Detection range: 0.0156-1 mg/mL		**Sensitivity:** 0.0078 mg/mL
	Applicable samples: Plant Tissues, Liquid samples such as Juice and Honey		
	Storage: Stored at 4℃ for 6 months, protected from light		

Assay Principle

Tannin is a kind of polyphenolic compounds widely present in plants, also known as plant polyphenols. It generally has astringent taste, can precipitate proteins, alkaloids, and polysaccharides, and can have complex or electrostatically interact with various metal ions. According to chemical structure, tannin can be divided into hydrolyzable tannin and condensed tannin. The ability of tannin binding to proteins is also known as astringency. Its astringency is the basis of various physiological activities, such as hemostasis, inhibition of microorganisms, anti-allergy, anti-mutation, anti-tumor, anti-aging and other physiological activities, and it is also one of the factors affecting the taste of products. CheKine™ Tannin Colorimetric Assay Kit provides a convenient tool for detection of Tannin. The principle is that tannin reacts with phosphomolybdic acid in an alkaline environment to form a blue compound, which has a characteristic absorption peak at 760 nm. The tannin content of the sample can be calculated by measuring the absorbance at 760 nm.

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	48 T	96 T	
Assay Buffer	5 mL	10 mL	4℃
Chromogen	5 mL	10 mL	4℃, protected from light
Standard	1	1	4℃, protected from light

Materials Required but Not Supplied

- Microplate Reader capable of measuring absorbance at 760 nm
- Incubator, Water Bath, Centrifuge
- 96 well plate with clear flat bottom, Precision Pipettes, Disposable Pipette Tips
- Deionized Water
- Homogenizer(for plant tissue with less fibers)
- Oven, pulverizer or wall breaker, 40-mesh sieve (for plant tissue with more fibers)

Reagent Preparation

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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.

Standard: Add 1 mL Deionized Water to dissolve before use. The concentration is 5 mg/mL. Store at 4℃, protected from light.

Standard curve setting: Dilute 5 mg/mL standard with Deionized Water as shown in the table below.

	Volume of Standard	Volume of Deionized Water (μL)	The concentration of Standard（mg/mL）
Std.1	200 μL of 5 mg/mL	800	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

Sample Preparation

1. Plant Tissues

(1) Pant Tissue with less fibers: Weigh 0.1 g tissue, add 1 mL Deionized Water and fully homogenize, then transfer to an EP tube, extract in a water bath at 80℃ for 30 min. Centrifuge at 8,000 g for 10 min at 25℃. Use supernatant for assay.

(2) Pant Tissue with more fibers: Sample was dried to constant weight, pulverized, passed through a 40-mesh sieve. Then weigh 0.1 g , add 1 mL Deionized Water mix well, extract in a water bath at 80℃ for 30 min. Centrifuge at 8,000 g for 10 min at 25℃. Use supernatant for assay.

2. Liquid samples such as Juice and Honey: Tested directly.

Assay Procedure

1. Preheat the microplate reader for more than 30 min, and adjust the wavelength to 760 nm.

2. Add the following reagents respectively into each well of 96-well plate：

	Blank well (μL)	Standard well (μL)	Test well (μL)
Deionized Water	100	90	90
Stds.	0	10	0
Sample	0	0	10
Chromogen	50	50	50
Assay Buffer	50	50	50

3. Mix well, incubate at room temperature (25℃) for 10 min. Then reading the values at 760 nm. Finally, calculate $\Delta A_{Test}=A_{Test}-A_{Blank}$, $\Delta A_{Standard}=A_{Standard}-A_{Blank}$. (Only one blank well needs to be detected).

Note: In order to guarantee the accuracy of experimental results, need to do a pre-experiment with 2-3 samples. If A_{Test} is greater than 2.0, the sample can be appropriately diluted with Deionized Water, 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. Substitute

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the ΔA_{Test} into the equation to obtain the y value (mg/mL).

2. Calculate the content of Tannin

(1) By sample weight

Tannin (mg/g)=$y \times V_{Extraction} \div W \times n$=**$y \div W \times n$**

(2) Calculated by liquid volume

Tannin (mg/mL)=**$y \times n$**

Where: $V_{Extraction}$: Deionized Water volume added for Sample extraction, 1 mL; W: sample weight, g; n: dilution multiple of sample.

Typical Data

Typical standard curve

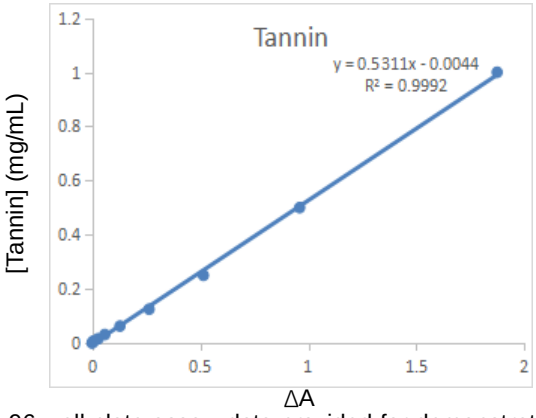

Figure1. Standard Curve of Tannin in 96-well plate assay–data provided for demonstration purposes only. A new standard Curve must be generated for each assay.

Examples

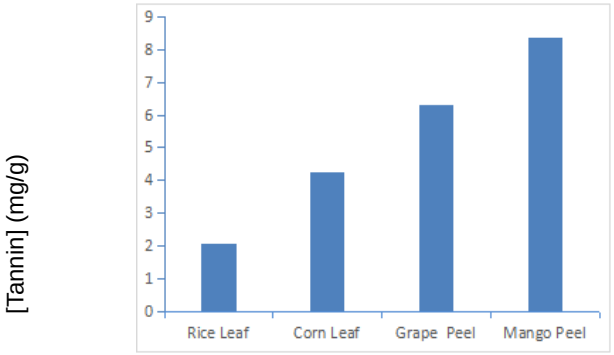

Figure 2. Tannin content in Rice Leaf, Corn Leaf, Grape Peel and Mango Peel respectively. Assays were performed following kit protocol

Recommended Products

Catalog No.	Product Name
KTB1540	CheKine™ Plant Total Phenols (TP) Colorimetric Assay Kit
KTB1530	CheKine™ Plant Flavonoids Colorimetric Assay Kit
KTB1520	CheKine™ Plant Oligomeric Proantho Cyanidins (OPC) Colorimetric Assay Kit

Disclaimer

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

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