（FOR RESEARCH USE ONLY. DO NOT USE IT IN CLINICAL DIAGNOSIS !）

Catalog No：E-BC-K820-M

Specification：48T (32 samples)/96T (80 samples)

Measuring instrument：Microplate reader(740-780 nm)

Detection range：0.41-250 mg/L

Elabscience® Tannins Colorimetric Assay Kit

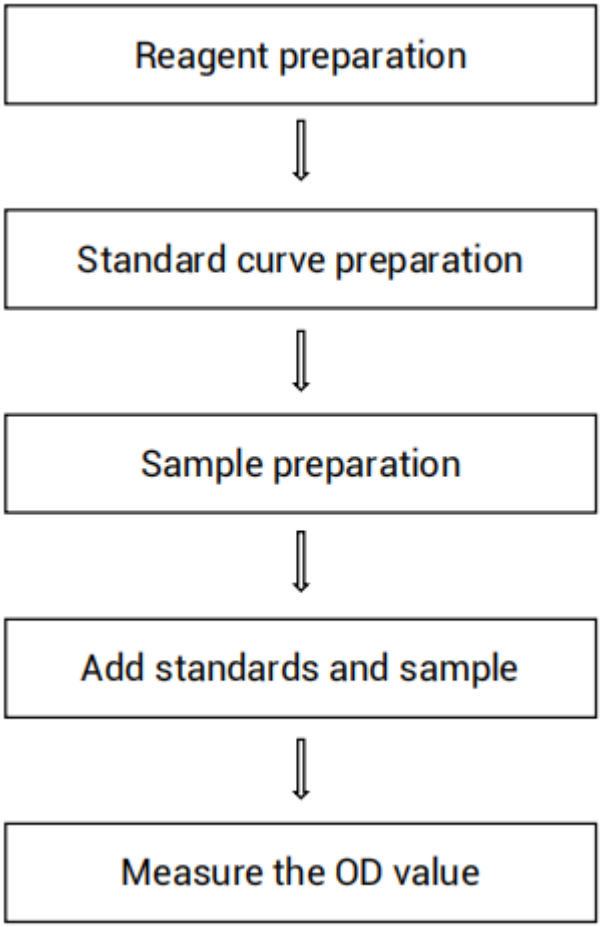
This manual must be read attentively and completely before using this product.
If you have any problem, please contact our Technical Service Center for help：

Toll-free: 1-888-852-8623
Tell: 1-832-243-6086
Fax: 1-832-243-6017
Email: techsupport@elabscience.com
Website: www.elabscience.com

Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

Table of contents

Assay summary



Intended use

This kit can be used to measure tannins content in juice and plant tissue samples.

Detection principle

Tannins are a class of natural polyphenolic compounds that are widely present in plants and have the property of binding with proteins, alkaloids and polysaccharides. With biological activities such as antioxidant, anti-inflammatory, and antibacterial effects, tannins have extensive applications in nutritional research, agricultural and plant sciences, and industrial fields.

This kit determines the tannin content in the sample by measuring the OD value at 760 nm of the blue compound produced by the reaction between the sample and the chromogenic reagent under alkaline conditions.

Kit components & storage

Item	Component	Size 1(48 T)	Size 2(96 T)	Storage
Reagent 1	Chromogenic Agent	3 mL × 1 vial	6 mL × 1 vial	2-8°C, 12 months, shading light
Reagent 2	Buffer Solution	6 mL × 1 vial	12 mL × 1 vial	2-8°C, 12 months
Reagent 3	2.5 g/L Standard Solution	1.6 mL × 1 vial	1.6 mL × 1 vial	2-8°C, 12 months, shading light
	Microplate	48 wells	96 wells	No requirement
	Plate Sealer	2 pieces		
	Sample Layout Sheet	1 piece		

Note: The reagents must be stored strictly according to the preservation conditions in the above table. The reagents in different kits cannot be mixed with each other. For a small volume of reagents, please centrifuge before use, so as not to obtain sufficient amount of reagents.

Materials prepared by users

Instruments:

Microplate reader (740-780 nm, optimum wavelength: 760 nm), Incubator

Reagents:

Double distilled water

Reagent preparation

① Equilibrate all the reagents to 25°C before use.

② The preparation of 250 mg/L standard solution:

Before testing, please prepare sufficient 250 mg/L standard solution. For example, prepare 1000 μL of 250 mg/L standard solution (mix well 100 μL of 2.5 g/L standard solution and 900 μL of double distilled water). Store at 2-8°C for 7 days protected from light.

③ The preparation of standard curve：

Always prepare a fresh set of standards. Discard working standard dilutions after use.

Dilute 250 mg/L standard solution with double distilled water to a serial concentration, the recommended dilution gradient is as follows: 0, 50, 100, 125, 150, 175, 200, 250 mg/L. Reference is as follows:

Item	①	②	③	④	⑤	⑥	⑦	⑧
Concentration (mg/mL)	**0**	**50**	**100**	**125**	**150**	**175**	**200**	**250**
250 mg/L standard (μL)	0	40	80	100	120	140	160	200
Double distilled water (μL)	200	160	120	100	80	60	40	0

Sample preparation

① Sample preparation

Liquid samples: detect directly.

Tissue sample:

① Harvest the amount of tissue needed for each assay (initial recommendation 20 mg).

② Homogenize 20 mg tissue in 180 μL double distilled water with a dounce homogenizer at 4°C.

③ Heat and extract in an 80°C water bath for 30 min, then cool to room temperature under running water.

④ Centrifuge at 10000×g for 10 min at 4°C to remove insoluble material. Collect supernatant for detection.

② Dilution of sample

The recommended dilution factor for different samples is as follows (for reference only):

Sample type	Dilution factor
Peach juice	2-6
Orange juice	3-8
Red wine	5-10
10% Blueberry tissue homogenate	2-5
10% Orange tissue homogenate	1
10% Orange peel tissue homogenate	2-4
10% Epipremnum aureum leaf tissue homogenate	1
10% Scandent schefflera leaf tissue homogenate	1

Note: The diluent is double distilled water. For the dilution of other sample types, please do pretest to confirm the dilution factor.

Operating steps

① Standard well: Add 20 μL of standard solution with different concentrations into the wells.
Sample well: Add 20 μL of samples into wells.

② Add 50 μL of double distilled water into each well.

③ Add 50 μL of chromogenic agent into each well.

④ Add 100 μL of buffer solution into each well.

⑤ Mix fully for 5 s and incubate at 37°C for 10 min protected from light.

⑥ Measure the OD value of each well at 760 nm with microplate reader.

Calculation

The standard curve:

1. Average the duplicate reading for each standard.

2. Subtract the mean OD value of the blank (Standard #①) from all standard readings. This is the absoluted OD value.

3. Plot the standard curve by using absoluted OD value of standard and correspondent concentration as y-axis and x-axis respectively. Create the standard curve (**y = ax + b**) with graph software (or EXCEL).

The sample:

Liquid samples:

$$\text{Tannins content (mg/L)} = (\Delta A - b) \div a \times f$$

Plant tissue samples:

$$\text{Tannins content (mg/g wet weight)} = (\Delta A - b) \div a \div \frac{m}{V} \div 1000* \times f$$

[Note]

ΔA: $\Delta A = OD_{sample} - OD_{blank}$.

m: The weight of tissue sample, g.

V: The volume of double distilled water in the preparation step of sample, mL.

1000*: 1 L = 1000 mL

f: Dilution factor of sample before test.

Appendix I Performance Characteristics

1. Parameter:

Intra-assay Precision

Three red wine samples were assayed in replicates of 20 to determine precision within an assay (CV = Coefficient of Variation).

Parameters	Sample 1	Sample 2	Sample 3
Mean (mg/L)	96.00	135.00	196.00
%CV	1.5	1.1	0.4

Inter-assay Precision

Three red wine samples were assayed 20 times in duplicate by three operators to determine precision between assays.

Parameters	Sample 1	Sample 2	Sample 3
Mean (mg/L)	104.00	139.00	248.00
%CV	4.4	0.7	1.3

Recovery

Take three samples of high concentration, middle concentration and low concentration to test the samples of each concentration for 6 times parallelly to get the average recovery rate of 95%.

	Sample 1	Sample 2	Sample 3
Expected Conc. (mg/L)	9.00	16.30	27.10
Observed Conc. (mg/L)	8.5	15.4	25.8
Recovery rate (%)	94.5	94.5	95.1

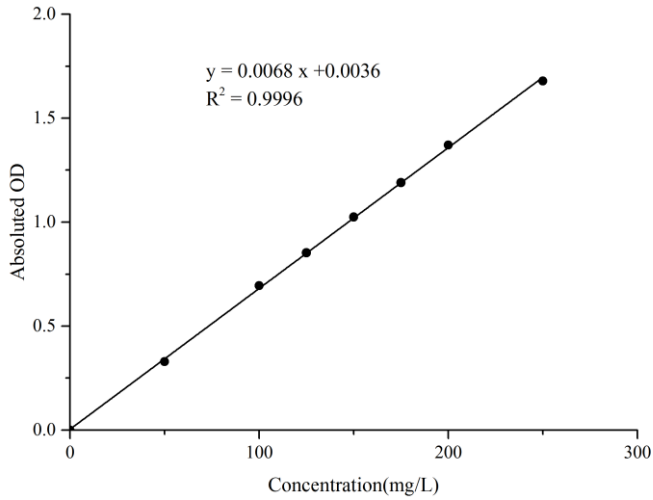
Sensitivity

The analytical sensitivity of the assay is 0.41 mg/L. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 20 times, and calculating the corresponding concentration.

2. Standard curve:

As the OD value of the standard curve may vary according to the conditions of the actual assay performance (e.g. operator, pipetting technique or temperature effects), so the standard curve and data are provided as below for reference only：

Concentration (mg/L)	0	50	100	125	150	175	200	250
OD value	0.043	0.371	0.741	0.889	1.068	1.252	1.443	1.733
	0.043	0.372	0.734	0.903	1.067	1.213	1.385	1.710
Average OD value	0.043	0.372	0.738	0.896	1.068	1.233	1.414	1.722
Absolute OD value	0	0.329	0.695	0.853	1.025	1.190	1.371	1.679



Appendix Π Example Analysis

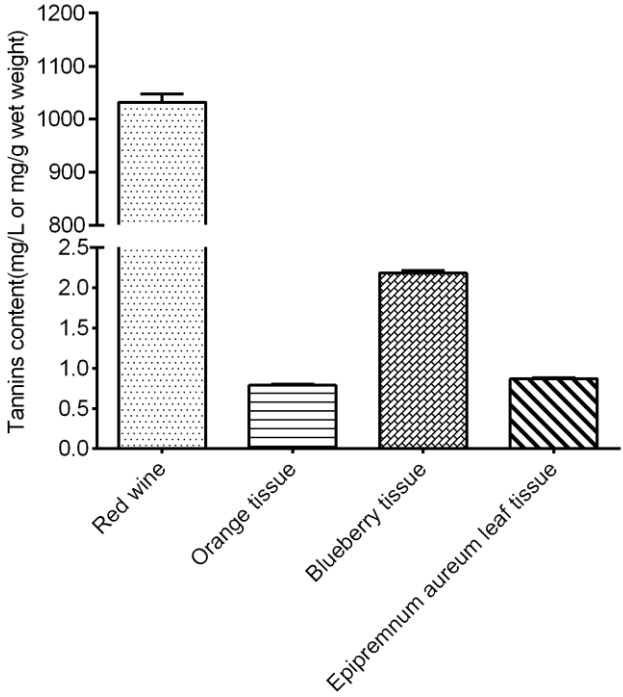
Example analysis：

Take 20 μL of red wine which dilute for 5 times and carry the assay according to the operation steps. The results are as follows:

Standard curve: y = 0.0068 x + 0.0036, the average OD value of the sample well is 1.451, the average OD value of the blank well is 0.043. The calculation result is:

Tannins content (mg/L) = (1.451 – 0.043 – 0.0036) ÷ 0.0068 × 5 = 1032.65 mg/L

Detect red wine (dilute for 5 times), 10% orange tissue homogenate and 10% blueberry tissue homogenate and 10% epipremnum aureum leaf tissue homogenate, according to the protocol, the result is as follows:



Statement

1. This assay kit is for Research Use Only. We will not response for any arising problems or legal responsibilities causing by using the kit for clinical diagnosis or other purpose.
2. Please read the instructions carefully and adjust the instruments before the experiments. Please follow the instructions strictly during the experiments.
3. Protection methods must be taken by wearing lab coat and latex gloves.
4. If the concentration of substance is not within the detection range exactly, an extra dilution or concentration should be taken for the sample.
5. It is recommended to take a pre-test if your sample is not listed in the instruction book.
6. The experimental results are closely related to the situation of reagents, operations, environment and so on. Elabscience will guarantee the quality of the kits only, and NOT be responsible for the sample consumption caused by using the assay kits. It is better to calculate the possible usage of sample and reserve sufficient samples before use.