



Tannin

Colorimetric Microplate Assay Kit

User Manual

Catalog # CAK1060

(Version 1.3D)

Detection and Quantification of Tannin Content in Tissue extracts,
Cell lysate Samples.

For research use only. Not for diagnostic or therapeutic procedures.

I. INTRODUCTION.....	2
II. KIT COMPONENTS.....	3
III. MATERIALS REQUIRED BUT NOT PROVIDED.....	4
IV. REAGENT PREPARATION.....	5
V. SAMPLE PREPARATION.....	6
VI. ASSAY PROCEDURE.....	7
VII. CALCULATION.....	8
VIII. TYPICAL DATA.....	9

I. INTRODUCTION

A tannin is an astringent, bitter plant polyphenolic compound that binds to and precipitates proteins and various other organic compounds including amino acids and alkaloids. The tannin compounds are widely distributed in many species of plants, where they play a role in protection from predation, and perhaps also as pesticides, and in plant growth regulation. The astringency from the tannins is what causes the dry and puckery feeling in the mouth following the consumption of unripened fruit or red wine. Likewise, the destruction or modification of tannins with time plays an important role in the ripening of fruit and the aging of wine.

Tannin Colorimetric Microplate Assay Kit provides a simple and direct procedure for measuring tannin content in a variety of samples. Tannin can react with phosphomolybdic acid, and the product can be measured at a colorimetric readout at 650 nm.

II. KIT COMPONENTS

Component	Volume	Storage
96-Well Microplate	1 plate	
Reaction Buffer	2 ml x 1	4 °C
Dye Reagent	1 ml x 1	4 °C, keep in dark
Standard	Powder x 1	4 °C, keep in dark
Plate Adhesive Strips	3 Strips	
Technical Manual	1 Manual	

III. MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at 650 nm
2. Distilled water
3. Pipettor, multi-channel pipettor
4. Pipette tips
5. Mortar
6. Centrifuge
7. Timer
8. Ice

IV. REAGENT PREPARATION

Standard: Briefly centrifuge prior to opening. Dissolve in 2 ml distilled water to generate 1 mg/ml of standard top solution, keep in dark and store at 4 °C for 1 week after reconstitution. Perform 2-fold serial dilutions of the top standard solution using distilled water to make the standard curve. The concentration of standard curve could be 1/0.5/0.25/0.125/0.0625/0.0312/0.0156 mg/ml.

V. SAMPLE PREPARATION

1. For tissue samples

Weigh out 0.1 g tissue, homogenize with 1 ml distilled water, put it in water bath of 80°C for 30 minutes, centrifuged at 8,000g 4°C for 10 minutes, take the supernatant into a new centrifuge tube for detection.

2. For liquid samples

Detect directly.

VI. ASSAY PROCEDURE

Warm the Reaction Buffer, Dye Reagent to room temperature before use.

Add following reagents into the microplate:

Reagent*	Sample**	Standard	Blank
Distilled water	160 μ l	160 μ l	170 μ l
Sample	10 μ l	--	--
Standard	--	10 μ l	--
Reaction Buffer	20 μ l	20 μ l	20 μ l
Mix, keep at room temperature for 5 minutes.			
Dye Reagent	10 μ l	10 μ l	10 μ l
Mix, wait for 10 minutes at room temperature, measured at 650 nm and record the absorbance.			

Note:

*Reagents must be added sequentially and should not be premixed prior to addition.

**The concentrations can vary over a wide range depending on the different samples.

For unknown samples, we recommend doing a pilot experiment & testing several doses to ensure the readings are within the standard curve range.

**For colored samples, we recommend setting a parallel sample background control well with same volume of sample only. Other reagents were replaced by distilled water to the same total volume. Subtract the OD value of the sample background control from the OD value of the sample to correct for interference from the sample's own color.

VII. CALCULATION

1. Calculate the sample concentration in ASSAY PROCEDURE according to the slope of the standard curve

$$C = \frac{(OD_{\text{Sample}} - OD_{\text{Blank}}) - \text{Intercept}}{\text{Slope}} \times n (\text{mg/ml})$$

Calculate the initial concentration according to sample preparation procedure.

2. According to one point of the standard OD and concentration

2.1 According to the protein concentration of sample

$$C = \frac{(C_{\text{Standard}} \times V_{\text{Standard}}) \times (OD_{\text{Sample}} - OD_{\text{Blank}})}{(OD_{\text{Standard}} - OD_{\text{Blank}}) \times C_{\text{Protein}} \times V_{\text{Sample}}} (\text{mg/mg})$$

2.2 According to the weight of sample

$$C = \frac{(C_{\text{Standard}} \times V_{\text{Standard}}) \times (OD_{\text{Sample}} - OD_{\text{Blank}})}{(OD_{\text{Standard}} - OD_{\text{Blank}}) \times W \times (V_{\text{Sample}} / V_{\text{Assay}})} (\text{mg/g})$$

2.3 According to the volume of sample

$$C = \frac{(C_{\text{Standard}} \times V_{\text{Standard}}) \times (OD_{\text{Sample}} - OD_{\text{Blank}})}{(OD_{\text{Standard}} - OD_{\text{Blank}}) \times V_{\text{Sample}}} (\text{mg/ml})$$

Slope: the absorbance slope of standard curve

n: the dilution factor

C_{Standard} : the standard concentration, mg/ml

V_{Standard} : the volume of standard in assay procedure, μl

V_{Sample} : the volume of sample in assay procedure, μl

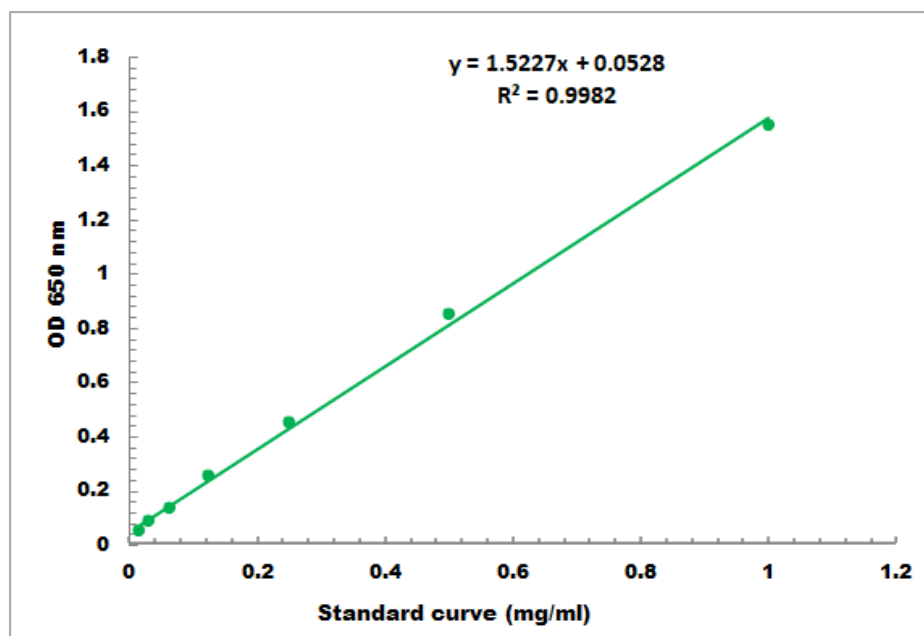
V_{Assay} : the volume of Assay Buffer, μl

C_{Protein} : the sample protein concentration, mg/ml

W: the weight of sample, g

VIII. TYPICAL DATA

The standard curve is for demonstration only. A standard curve must be run with each assay.



Detection Range: 0.01mg/ml - 1mg/ml