Tannin Microplate Assay Kit

User Manual

Catalog # CAK1060

(Version 1.2D)

Detection and Quantification of Tannin Content in Tissue extracts,

Cell lysate Samples.

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

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 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
Standard	Powder x 1	4 °C
Plate Adhesive Strips	3 Strips	
Technical Manual	1 Manual	

Note:

Standard: add 2 ml distilled water to dissolve before use, the concentration will be 1 mg/ml.

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. 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.

V. ASSAY PROCEDURE

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

Add following reagents into the microplate:

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

Note:

1) Perform 2-fold serial dilutions of the top standards to make the standard curve.

2) 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.

3) Reagents must be added step by step, can not be mixed and added together.

VI. CALCULATION

1. According to the volume of sample

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

$$= (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank})$$

2. According to the weight of sample

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

$$= (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / W$$

$C_{Standard}$: the standard concentration, 1 mg/ml;

W: the weight of sample, g;

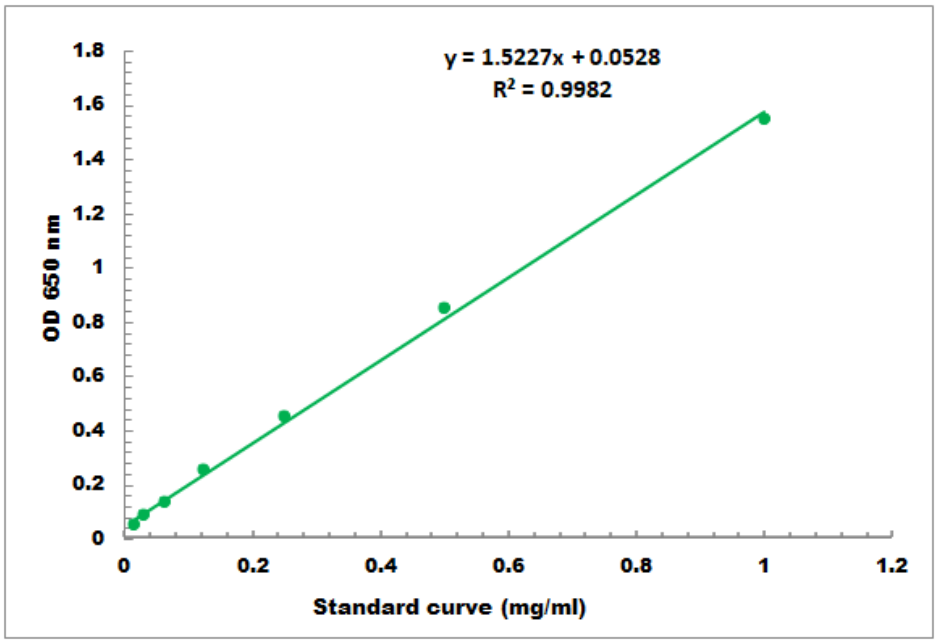
V_{Water}: the volume of distilled water, 1 ml;

V_{Sample}: the volume of sample, 0.01 ml;

$V_{Standard}$: the volume of sample, 0.01 ml.

VII. TYPICAL DATA

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



Detection Range: 10 µg/ml - 1000 µg/ml

VIII. TECHNICAL SUPPORT

For troubleshooting, information or assistance, please go online to www.cohesionbio.com or contact us at techsupport@cohesionbio.com

IX. NOTES