Tannin Assay Kit

Catalog No.: abx298826

Size: 100 Assays

Storage: Store all kit components at 4°C.

Application: For quantitative detection of Tannin concentrations in tissue homogenates and cell lysates.

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

Introduction: Tannins are a group of bitter and astringent polyphenols that bind to and precipitate proteins, amino acids, alkaloids, and various other organic compounds. Tannins are widely found in many plant species, and act as a deterrent from predators. The destruction or modification of tannins plays an important role in the ripening of fruit and aging of wine.

Abbexa's Tannin Assay Kit is a quick, convenient, and sensitive method for measuring and calculating Tannin content. Tannin reacts with phosphomolybdic acid to produce an absorbance at 650 nm. The intensity of the color is proportional to the concentration of Tannin, which can then be calculated.

Kit components

1. 96 well microplate
2. Reaction Buffer: 2 ml
3. Dye Reagent: 1 ml
4. Standard: 1 vial

Materials Required But Not Provided

1. Microplate reader (650 nm)
2. Centrifuge and microcentrifuge tubes
3. High-precision pipette and sterile pipette tips
4. Distilled water
5. Timer
6. Sonicator
7. Mortar
8. Water bath

Protocol

A. Preparation of Sample and Reagents

1. Reagents

- **Standard Solution**
  Add 2 ml of distilled water to the Standard vial and mix thoroughly to prepare a 1 ml Standard Solution with concentration 1 mg/ml (1000 µg/ml). Ensure that the Standard has completely dissolved prior to use.

2. Samples

- **Tissue samples**
  Homogenize 0.1 g of sample in 1 ml of distilled water. Heat the homogenate in a water bath set to 80°C for 30 minutes. Centrifuge at 8000 × g at 4°C for 10 minutes. Transfer the supernatant to a new tube, keep on ice and analyze immediately.

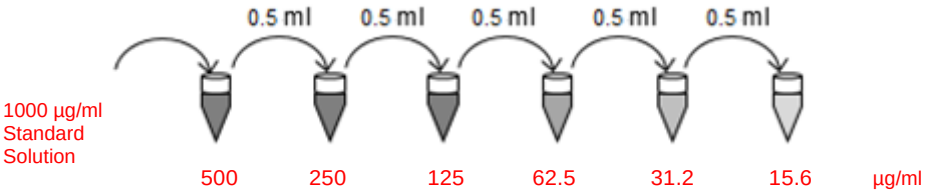
- **Liquid samples**
  Liquid samples can be used directly.

B. Assay Procedure

Bring all reagents to room temperature prior to use.

If the expected concentration is unknown, a preliminary experiment is recommended to determine if sample dilutions or adjustments to the reaction time are required to bring the measured concentrations within the detection range of the kit.

1. Label 6 tubes with 500 µg/ml, 250 µg/ml, 125 µg/ml, 62.5 µg/ml, 31.2 µg/ml, and 15.6 µg/ml. Aliquot 0.5 ml of distilled water into each tube. Add 0.5 ml of 1000 µg/ml Standard Solution to the 1st tube and mix thoroughly. Transfer 0.5 ml from the 1st tube to the 2nd tube and mix thoroughly, and so on.



2. Set the sample, standard and blank wells on the 96-well microplate and record their positions. We recommend setting up each standard and sample in duplicate.
3. Add 10 µl of sample to the sample wells.
4. Add 10 µl of prepared standards to the standard wells.
5. Add 160 µl of distilled water to the sample wells and the standard wells.
6. Add 170 µl of distilled water to the blank wells.
7. Add 20 µl of Reaction Buffer to each well.
8. Tap the plate gently to mix. Allow to stand at room temperature for 5 minutes.
9. Add 10 µl of Dye Reagent to each well.
10. Tap the plate gently to mix. Allow to stand at room temperature for 10 minutes.
11. Read and record absorbance at 650 nm.

C. Calculations

Tannin concentration per ml of sample:

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

Tannin concentration per g of sample:

$$\textbf{Tannin (mg/g sample)} = \frac{C_{Standard} \times V_{Standard} \times V_{Water}}{V_{Sample} \times W} \times \frac{OD_{Sample} - OD_{Blank}}{OD_{Standard} - OD_{Blank}} = \frac{1}{W} \times \frac{OD_{Sample} - OD_{Blank}}{OD_{Standard} - OD_{Blank}}$$

where:

$C_{Standard}$	Concentration of highest standard (1 mg/ml = 1000 µg/ml)
V_{Water}	Volume of distilled water (1 ml)
V_{Sample}	Volume of sample (0.01 ml)
$V_{Standard}$	Volume of standard (0.01 ml)
W	Weight of the sample (in g)