Plant Flavonoids Assay Kit

Catalog No.: abx298941

Size: 96 tests

Detection Range: 0.66 µg/ml – 150 µg/ml

Sensitivity: 0.66 µg/ml

Storage: Store all components at 4ºC.

Application: For detection and quantification of Plant Flavonoids content in plant tissue samples.

Introduction

Abbexa's Plant Flavonoids Assay Kit is a quick, convenient, and sensitive method for measuring and calculating Plant Flavonoids content in plant tissue samples. The product has an absorbance maximum at 510 nm. When suspended in alkaline nitrite solution, Plant Flavonoids form a red-coloured complex with Aluminium ions. The concentration of Plant Flavonoids in samples is calculated by measuring absorbance at 510 nm.

Kit components

1. 96-well UV microplate
2. Standard solution (1 mg/ml): 1.8 ml
3. Saline solution: 2 × 1 ml
4. Aluminium reagent: 2 × 1.8 ml
5. Alkaline nitrite reagent: 30 ml
6. Plate sealer: 2

Materials Required But Not Provided

1. Microplate reader (510 nm)
2. Micropipette and pipette tips
3. Vortex mixer
4. Centrifuge
5. Vacuum dryer
6. Sonicator
7. Double-distilled water
8. 60% Ethanol
9. 100% Ethanol

Protocol

A. Preparation of samples and reagents

1. Samples

- **Plant tissues**: Weigh 5 – 10 g of fresh plant tissue and wash with distilled water. Dry samples using tissue paper with filter paper as an intermediary, then dry completely in vacuum dryer at 80ºC until constant weight is recorded. Crush dried sample and filter at room temperature in a sealed environment, though a 40-mesh filter screen. Weigh 0.02 g of crushed and dried sample, suspend in 2 ml of 60% Ethanol, then incubate at 60ºC in a shaking incubator for 2 hours. Centrifuge at 1500 × g for 10 min, then collect the supernatant using a pipette. Alternatively, sonicate samples (300W, 3 s duration, 4 s interval, 30 minutes), then centrifuge at 10,000 × g for 10 min and take the supernatant for detection.

It is recommended to carry out a preliminary experiment to determine the optimal dilution factor of samples before carrying out the formal experiment. Dilute samples so that they fall within the detection range of the assay with 60 % Ethanol.

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

Sample Type	Dilution Factor
Epipremnum aureum	10 – 15
Green pepper	1
Pumpkin	1
Heather	25 – 35

Notes:

- Fresh samples or recently obtained samples are recommended to prevent degradation and denaturalization that may lead to erroneous results.

2. Reagents

Bring all reagents to room temperature before use.

B. Assay Procedure

1. Set standard and sample to wells on the 96-well microplate and label accordingly.
2. Dilute 1 mg/ml standard solution with 100% ethanol according to the following table. The recommended dilution gradient is 0, 20, 40, 60, 80, 100, 120, and 150 μg/ml.

	Standard Concentration (μg/ml)	1 mg/ml Standard Solution (μl)	100% Ethanol Diluent (μl)
A	0	0	1200
B	20	24	1176
C	40	48	1152
D	60	72	1128
E	80	96	1104
F	100	120	1080
G	120	144	1056
H	150	180	1020

3. **Standard Well:** Add 75 μl each standard solution to the corresponding standard wells.
4. **Sample Well:** Add 75 μl prepared sample to the sample wells.
5. Add 10 μl saline solution to each well, mix and incubate for 5 min at room temperature.
6. Add 30 μl Aluminium reagent to each well, mix and incubate for 5 min at room temperature.
7. Add 180 μl Alkaline nitrite reagent to each well, mix and incubate for 15 min at room temperature.
8. Measure the OD value of each well at 510 nm using a microplate reader.

C. Calculation of Results

Plot the linear standard curve where x = concentration and y = OD measurement, producing a curve with the formula $y = ax + b$.

$$Flavonoid\ concentration\ (mg/g) = \frac{\Delta A_{510} - b}{a} \times \frac{V}{W \times 1000} \times f$$

where:

y	$\mathrm{OD}_{\mathrm{Standard}} - \mathrm{OD}_{\mathrm{Blank}}$
x	Concentration of Standard
a	Slope of standard curve
b	Intercept of standard curve
ΔA_{510}	$\mathrm{OD}_{\mathrm{Sample}} - \mathrm{OD}_{\mathrm{Blank}}$
V	Volume of 60% ethanol in sample pretreatment (2 ml)
W	Weight of sample (0.02 g)
1000	Unit conversion (1000 μg = 1 mg)
f	Dilution factor samples prior to assay