Flavonoids Assay Kit

Catalog No.: abx295067

Size: 100 tests

Detection Range: 0.315 µg/ml – 150 µg/ml

Sensitivity: 0.315 µg/ml

Storage: Store all components at 4°C.

Application: For detection and quantification of Flavonoids concentration in plant tissue homogenates.

Introduction

Flavonoids are a diverse group of naturally occurring polyphenolic compounds commonly found in plants. Structurally, they are characterized by two aromatic benzene rings connected by a three-carbon bridge. compounds are well-known for their potent antioxidant activity, primarily due to their ability to donate hydrogen atoms or electrons to neutralize reactive oxygen species and prevent oxidative stress. Beyond this, flavonoids exhibit significant anti-inflammatory, anti-carcinogenic, and cardioprotective properties. As such, they have garnered interest for their potential therapeutic applications.

Abbexa's Flavonoids Assay Kit is a quick, convenient, and sensitive method for measuring and calculating flavonoids concentration. In an alkaline nitrate solution, flavonoids combine with aluminum ions to produce a red compound. This compound has an absorbance maximum at 510 nm. The intensity of the color is proportional to the Flavonoids concentration, which can then be calculated.

Kit components

1. Saline Solution: 4 ml
2. Aluminum Reagent: 4 ml
3. Alkaline Reagent: 50 ml
4. Standard (1 mg/ml): 2 ml

Materials required but not provided

1. Spectrophotometer (510 nm)
2. Double-distilled water
3. 60% ethanol
4. Absolute ethanol
5. Vacuum dryer
6. Ultrasonicator
7. Pipette and pipette tips
8. 2 ml microcentrifuge tubes
9. Centrifuge
10. Vortex mixer
11. Incubator
12. Filter paper
13. Size 40 mesh screen

Protocol

A. Preparation of samples and reagents

1. Samples

Isolate the test samples soon after collecting and analyze immediately or aliquot and store at -20°C or -80°C for long-term storage. Avoid multiple freeze-thaw cycles.

The following sample preparation method is intended as a guide and may be adjusted as required depending on the specific samples used.

Drying: Carefully weigh 5-10g of fresh plant tissue and wash in double-distilled water. Absorb surface moisture from sample using filter paper before placing in a 80°C vacuum dryer to completely dry. Crush dried sample and filter through a 40-mesh filter screen, at room temperature in a sealed environment.

Extraction: Accurately weigh 20 mg of dried sample into a clean microcentrifuge tube and add 2 ml 60% ethanol. Mix thoroughly using a vortex mixer for 2 hours at 60°C. Centrifuge at 1,500 × g for 10 minutes and take the supernatant for detection. *Alternatively*, homogenize using an ultrasonicator (power: 300 W, 3 seconds/time, interval for 4 seconds and repeat for 30 minutes) and centrifuge the homogenate at 10,000 × g for 10 minutes. 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 into different concentrations with 60% ethanol, then carry out the assay procedure. The recommended dilution factor for different samples is as follows (for reference only):

Sample Type	Dilution Factor
Camphor leaves tissue homogenate	8 – 15
Green pepper tissue homogenate	1
Carrot tissue homogenate	2 – 5

Note:

- Fresh samples or recently obtained samples are recommended to prevent degradation and denaturalization that may lead to erroneous results.
- Lysis buffers may interfere with the kit. It is therefore recommended to use mechanical lysis methods for tissue homogenates.

2. Reagents

- **Standards:** Prepare the standard dilutions according to the volumes summarized in the following tabl

Standard Dilution (µg/ml)	0	20	60	80	100	120	150
1 mg/ml Standard (µl)	0	24	72	96	120	144	180
Absolute ethanol (µl)	1200	1176	1128	1104	1080	1056	1020

For the blank, or 0 µg/ml standard, use pure absolute ethanol. The volume of each standard will be 1200 µl.

Note:

- Allow all reagents to equilibrate to room temperature before use.

B. Assay Procedure

1. Label microcentrifuge tubes for each standard and sample. *It is strongly recommended to prepare all the tubes in duplicate.*
2. Add 540 µl of each standard dilution to the corresponding tubes.
3. Add 540 µl of each sample to the corresponding tubes.
4. Add 30 µl of Saline Solution to all tubes. Mix thoroughly and incubate at room temperature for 5 minutes.
5. Add 30 µl of Aluminum Reagent into all tubes. Mix thoroughly and incubate at room temperature for 5 minutes.
6. Add 400 µl of Akali Reagent to all tubes. Mix thoroughly and incubate at room temperature for 15 minutes.
7. Zero the spectrophotometer using double-distilled water.
8. Measure the OD of each tube with a spectrophotometer at 510 nm using a 0.5 cm optical path cuvette.

C. Calculation of Results

Plot the standard curve, using the OD of the standard dilutions (adjusted for the blank) on the y-axis, and their respective concentrations on the x-axis. The curve should form a straight line, described by the formula $y = ax + b$. Based on this curve, the concentration of Flavonoids in each sample well can be derived with the following formula:

Plant tissue samples:

$$\text{Flavonoids Concentration (mg/g tissue)} = \mathrm{F} \times \frac{(\Delta\mathrm{A} - \mathrm{b})}{\mathrm{a}} \times \frac{\mathrm{V}}{\mathrm{W} \times 1000}$$

where:

ΔA	$OD_{Sample} - OD_{Blank}$
V	Volume of 60% ethanol in sample preparation (ml)
a	Gradient of the standard curve ($y = \boldsymbol{a}x + b$)
b	Y-intercept of the standard curve ($y = ax + \boldsymbol{b}$)
W	The weight of the sample (0.02 g)
1000	Unit conversion (1000 µg = 1 mg)
F	The dilution factor of sample

Technical Support

For troubleshooting and technical assistance, please contact us at support@abbexa.com.