Total Phenols Assay Kit

Catalog No.: abx294157

Size: 100 tests

Detection Range: 0.73 µg/ml– 150 µg/ml

Sensitivity: 0.73 µg/ml

Storage: Store all components at 4°C in the dark.

Application: For the detection and quantification of Total Phenols concentration in plant tissue homogenates.

Principle of the Assay

Abbexa's Total Phenol Assay Kit is a quick, convenient, and sensitive method for measuring and calculating Total Phenol concentration. Under alkaline conditions, tungsten-molybdenum acid is reduced by phenols to produce a blue compound with an absorbance maximum at 760 nm. The intensity of the color is proportional to the Total Phenol concentration, which can then be calculated.

Kit components

1. Folin Phenol Reagent: 60 ml
2. Alkaline Reagent: 2 vials
3. Standard: 4 × 10 mg

Materials required but not provided

1. Spectrophotometer (760 nm)
2. Double-distilled water
3. 60% Ethanol
4. Pipette and pipette tips
5. Centrifuge and centrifuge tubes
6. Sonicator
7. Mechanical homogenizer
8. Vortex mixer
9. Incubator
10. Vacuum dryer
11. 40 mesh sieve
12. 0.5 cm optical path cuvettes

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Protocol

A. Preparation of samples and reagents

1. Reagents

- **Alkaline Working Solution:** Reconstitute 1 vial Alkaline Reagent with 50 ml of double-distilled water. Mix thoroughly, until fully dissolved. Store unused Alkaline Working Substrate Solution at 4°C for up to 1 month in the dark.
- **1 mg/ml Standard:** Reconstitute 1 vial of Standard with 10 ml double-distilled water. As this standard is easily oxidized, prepare for immediate use.
- **Standards:** Label 6 tubes with 20 µg/ml, 40 µg/ml, 80 µg/ml, 100 µg/ml, 120 µg/ml, and 150 µg/ml. Prepare these dilutions according to the volumes in the following table:

Standard Dilution (µg/ml)	20	40	80	100	120	150
1 mg/ml Standard (µl)	20	40	80	100	120	150
Double-distilled water (µl)	980	960	920	900	880	850

For the blank, use pure double-distilled water. The volume of each standard dilution will be 1000 µl.

Note:

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

2. 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 methods are intended as a guide and may be adjusted as required depending on the specific samples used.

- **Plant tissue homogenates:** Carefully weigh at least 5 g of tissue. Wash the tissue with double-distilled water and pat dry using a filter paper. Transfer to a vacuum drying oven and dry at 40°C until a constant weight is reached. Crush dried tissue and pass through a 40 mesh sieve. Per 0.1 g of crushed sample, add into 2.5 ml of 60% Ethanol. Homogenize the sample using a sonicator set to 300 W, applying 3-second pulses with 4-second intervals for a total of 30 minutes. Centrifuge at 10,000 × g for 10 minutes at room temperature. Carefully take the supernatant for detection and assay immediately.

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 performance of this kit. It is recommended to use mechanical lysis methods only for tissue homogenates.

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B. Assay Procedure

1. Label centrifuge tubes for each standard, blank, sample, and control. *Each sample requires a corresponding control. It is strongly recommended to test all tubes in duplicate*.
2. Add 100 µl of each standard dilution to the standard tubes.
3. Add 100 µl of double-distilled water to the blank tubes.
4. Add 100 µl of sample to the sample tubes.
5. Add 100 µl of sample to the corresponding control tubes.
6. Add 500 µl of Folin Phenol Reagent to the blank, standard and sample tubes. Vortex tubes to mix thoroughly and stand at room temperature for 2 minutes.
7. Add 500 µl of Alkaline Working Solution and 900 µl of double-distilled water into the standard and sample tubes.
8. Add 500 µl of Alkaline Working Solution and 1400 µl of double-distilled water into the control tubes.
9. Vortex tubes to mix thoroughly and stand at room temperature for 10 minutes.
10. Set the spectrophotometer to 0 with double-distilled water and measure the OD of each tube at 760 nm using a 0.5 cm optical path cuvette.

C. Calculation of Results

Plot the standard curve, using the mean 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 Total Phenol in each sample well can be derived using the following formula:

1. Tissue samples:

$$\text{Total Phenol (U/mg protein)} = \frac{(\Delta A - b) \times V}{a \times W \times 1000} \times F$$

where:

ΔA	$OD_{Sample} - OD_{Control}$
V	Volume of 60% Ethanol (2.5 ml)
a	Gradient of the standard curve ($y = \boldsymbol{a}x + b$)
b	Y-intercept of the standard curve ($y = ax + \boldsymbol{b}$)
W	Weight of the tissue sample (0.1 g)
F	Dilution factor of the sample
1000	Unit conversion µg → mg

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Technical Support

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