# Total Phenols Assay Kit

**Catalog No.:** abx298934

**Size:** 96 tests

**Detection Range:** 1.05 µg/ml - 148 µg/ml

**Sensitivity:** 1.05 µg/ml

**Storage:** Store all components in the dark at 4°C for up to 12 months.

**Application:** For detection and quantification Total Phenols in plant tissue homogenates.



## Introduction

Phenols are a group of volatile small molecules that are commonly present as a secondary metabolite in plants. Phenolics are formed by three different biosynthetic pathways: the shikimate pathway, the polyketide pathway, and the acetate/mevalonate pathway. Phenols are reducing compounds and act as antioxidants inhibiting oxidation reactions and the generation of oxygen free radicals. These compounds are involved in a wide range of functions, such as the control of metabolism, defense against damage from Ultra-violet light, or attracting pollinators. Phenols can also serve as a defense against herbivores, attracting the predators of herbivores that attack the plant. In response to infection, many plants produce phenol compounds that signal to neighboring plants that a pathogen is present in the area. This can lead to the proactive upregulation of the hypersensitive response, and production of pathogen-inhibiting phenolic compounds prior to infection.

Abbexa's Total Phenols Assay Kit is a quick, convenient, and sensitive method for measuring and calculating Total Phenol concentration. Tungsten-molybdenum acid can be reduced by phenols to produce blue compounds with a characteristic absorption peak at 760 nm. The absorbance should be measured at 760 nm. The intensity of the color is proportional to the concentration of the Total Phenols, which can then be calculated.

### Kit components

1. 96-well microplate
2. Chromogenic Reagent: 10 ml
3. Alkali Reagent: 1 vial
4. Standard: 2 vials
5. Plate sealer: 2

### Materials Required But Not Provided

1. Microplate reader (760 nm)
2. Centrifuge
3. Double distilled water
4. Ethanol (60%)
5. Filter paper
6. Orbital shaker
7. Pipette and pipette tips
8. Sonicator
9. Timer
10. Vacuum drying oven
11. Vials/tubes
12. Weighing scales

## Protocol

### A. Preparation of samples and reagents

#### 1. Samples

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:** Collect 5-10 g of fresh plant tissue, and record the weight for later use (W). Rinse the surface with distilled water, and dry with filter paper. Place the plant tissue in a vacuum drying oven at 80°C. As the sample dries, the weight will decrease. Continue drying the sample until the weight no longer changes. Allow the sample to cool to room temperature, then crush and seal in an airtight container. Take 40 mg of crushed sample into a centrifuge tube. Add 1 ml of Ethanol (60%), and homogenize by hand, using a mechanical homogenizer, or by ultrasonication. Centrifuge the homogenate at 10,000 × g at room temperature for 10 min. Collect the supernatant and assay immediately.

Samples should not contain reducing agents such as DTT.

A preliminary experiment should be carried out to determine the optimal dilution factor of samples before carrying out the formal experiment. Where dilutions are required, samples should be diluted using Ethanol (60%).

| Sample type | Dilution factor |
|---|---|
| Epipremnum aureum tissue homogenate | 20-30 |
| Daucus carota tissue homogenate | 5-15 |
| Spinacia oleracea tissue homogenate | 15-25 |
| Leek tissue homogenate | 10-20 |

**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

Bring all reagents to room temperature before use.

- **Alkali Reagent Working Solution:** Add 10 ml of double distilled water to 1 vial of Alkali Reagent. Ensure the mixture has fully dissolved before carrying out the assay. **Note:** *This solution can be stored at 4°C for up to 1 month.*
- **1000 µg/ml Standard Solution:** Add 10 ml of double distilled water to 1 vial of Standard to prepare the 1000 µg/ml Standard Solution. Ensure the mixture has fully dissolved before carrying out the assay. **Note:** *This solution can be stored at 4°C in the dark for up to 1 month.*

### B. Assay Procedure

1. **Standard curve preparation:** Label 8 tubes with 150, 120, 100, 80, 60, 40 and 20 and 0 µg/ml. Add the following volumes of 1000 µg/ml standard solution to each tube respectively: 150, 120, 100, 80, 60, 40, 20, and 0 µl. Add the following volumes of double distilled water to the corresponding tubes: 1000, 980, 960, 940 920, 900, 880, and 850 µl.

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Concentration | 150 | 120 | 100 | 80 | 60 | 40 | 20 | 0 | µg/ml |
| 1000 µg/ml Standard Solution | 150 | 120 | 100 | 80 | 60 | 40 | 20 | 0 | µl |
| Double distilled water | 850 | 880 | 900 | 920 | 940 | 960 | 980 | 1000 | µl |

2. **Microplate procedure**

2.1. Set the Standard, Sample and Control wells, and record the positions. We recommend measuring each standard in duplicate. Each sample should have at least one corresponding control well.
2.2. Add 10 µl of each pre-prepared standard solution to the corresponding Standard wells (150, 120, 100, 80, 60, 40 and 20 and 0 µg/ml).
2.3. Add 10 µl of each sample to the corresponding sample wells.
2.4. Add 10 µl of each sample to the corresponding control wells.
2.5. Add 50 µl of Chromogenic Reagent to the sample and standard wells.
2.6. Add 50 µl of double distilled water to the control wells.
2.7. Mix by tapping the plate gently, or using an orbital shaker for 5 seconds.
2.8. Allow to stand at room temperature for 2 minutes.
2.9. Add 50 µl of Alkali Reagent working solution, and 90 µl of double distilled water to all wells.
2.10. Mix by tapping the plate gently, or using an orbital shaker for 5 seconds.
2.11. Allow to stand at room temperature for 10 minutes.
2.12. Measure the OD values at 760 nm with a microplate reader.

### C. Calculation of Results

The standard curve can be plotted as the corrected $OD_{760}$ of each standard solution, y ($OD_{Sample} - OD_{Blank}$) against the respective concentration of the standard solution ($x$). A linear fit is recommended for the standard curve (y = $ax$ + $b$). The Total Phenols content of the samples can be interpolated from the standard curve.

**1. Plant tissue homogenate samples:**

The content of Total Phenols per gram of wet tissue:

$$\text{Total Phenols (mg/g)} = \frac{\Delta A_{760} - b}{a} \times \frac{V \times f}{1000 \times W}$$
$$= \frac{\Delta A_{760} - b}{a} \times \frac{f}{40}$$

where:

| | |
|---|---|
| $\text{Total Phenols}$ | concentration of Total Phenols |
| $OD_{Blank}$ | average OD value of the 0 mg/ml standard wells |
| $OD_{Sample}$ | OD value of a sample |
| $OD_{Control}$ | OD value of a sample control |
| y | $OD_{Sample} - OD_{Blank}$ |
| x | Concentration of standard |
| $a$ | gradient of the standard curve (linear fit) |
| $b$ | y-intercept of the standard curve (linear fit) |
| $\Delta A_{760}$ | $OD_{Sample} - OD_{Control}$ |
| $f$ | dilution factor of the sample before carrying out the assay |
| V | volume of added Ethanol (60%) = 1 ml |
| W | wet weight of sample, recorded during sample preparation, 0.04 g |
| $1000$ | unit conversion, 1000 µg = 1 mg |

**Precision:**

Intra-assay Precision (Precision within an assay): samples with low, medium and high levels of phenol were tested on one plate.

Inter-assay Precision (Precision between assays):samples with low, medium and high levels of phenol were tested on multiple plates.

CV (%) = (Standard Deviation / Mean) × 100

Intra-Assay: CV < 5%

Inter-Assay: CV < 5%