



Phenolic Compounds Assay Kit (Colorimetric)

2/18

(Catalog # K527-200; 200 assays; Store at -20°C)

I. Introduction:

Phenolic compounds are phytochemical secondary metabolites found abundantly in dietary and medicinal plants, vegetables and fruits. Major types of phytochemical phenolic compounds include simple phenolic acids (such as gallic acid and vanillic acid), flavonoids (such as catechin), stilbenoids, lignans and various highly complex polyphenols (proanthocyanidins and tannins). These compounds play an important role in plant defense against ultraviolet radiation, serve as a deterrent to herbivores and also act as signaling molecules in ripening and other plant growth processes. Both simple phenolic acids and complex polyphenols are found in high concentrations in foods and beverages such as berries, vegetables, cereals, coffee, tea and wine. Phenolic compounds, being antioxidants, have been increasingly studied in dietary sources, due to their protective effects against cardiovascular diseases, cancer and neurodegenerative diseases. Studies have also shown dietary polyphenols to possess antimicrobial, anti-inflammatory and anti-allergic properties. BioVision's Phenolic Compounds Assay Kit provides a quick, sensitive and selective method for measuring the total amount of phenolic compounds in various biological samples. In this assay, phenolic compounds couple with diazonium salts under alkaline conditions to form a stable diazo chromophore, detectable by absorbance at 480 nm. Unlike the classical Folin-Ciocalteu (FC) protocol for measuring phenolic compounds, our assay is not affected by non-phenolic reducing substances such as sulfites, reducing sugars or ascorbic acid. The assay is high-throughput adaptable and can detect concentrations of phenolic compounds as low as 0.02 mM catechin equivalents (CEs) from a variety of plant and food-based samples.



II. Applications:

- Measurement of phenolic compounds in fruits/vegetables, beverages, food products, plants.

III. Sample Type:

- Fruits, vegetables; beverages (e.g. tea, wine, coffee); food products
- Plant extracts
- Natural or herbal products

IV. Kit Contents:

Components	K527-200	Cap Code	Part Number
PC Assay Buffer	25 ml	WM	K527-200-1
PC Probe	4 ml	Amber	K527-200-2
Catechin Standard (100 mM)	100 μ l	Yellow	K527-200-3
Vanillic Acid (50 mM)	500 μ l	Violet	K527-200-4

V. User Supplied Reagents and Equipment:

- 96-well clear plate with flat bottom
- Multi-well microplate spectrophotometer (ELISA reader)
- Reagent-grade (200 proof) ethanol
- Organic solvents (e.g. methanol, acetone) and dilute hydrochloric acid (1N HCl), for sample extraction

VI. Storage Conditions and Reagent Preparation:

Store kit components at -20°C, protected from light. Briefly centrifuge small vials prior to opening. Read entire protocol before performing the assay.

- **PC (Phenolic Compounds) Assay Buffer:** Store at -20°C. Warm to room temperature and vortex well before use.
- **PC (Phenolic Compounds) Probe:** Store at -20°C, protected from light. Warm to room temperature before use.
- **Catechin Standard (100 mM):** Aliquot and store at -20°C, protected from light. Close cap tightly. Use within two months.
- **Vanillic Acid (50 mM):** Aliquot and store at -20°C. Close cap tightly, protected from. Use within two months.

VII. Phenolic Compounds Assay Protocol:

1. Sample Preparation:

- a. A variety of fruit, vegetable and plant samples, beverages as well as herbal/natural products can be analyzed with this assay. Fruit, vegetable and plant extractions can be performed using acid/methanol (for example, using a 70:29.5:0.5 ratio solution of Methanol:ddH₂O:1N HCl), acid/ethanol or acetone extraction methods. Users may use the extraction methods of their choice for their particular samples (which may vary based upon the sample type), with proper dilutions to ensure the values fall within the standard curve range. **For more details about various phenolic extraction methods, see References 1-2.** Fruit/vegetable juices, liquid herbal products and freeze-dried fruits solubilized in suitable solvents, beverages such as wines, green tea, and coffee can also be used directly with appropriate dilutions, while making sure potential interfering substances do not give a significant background. **Chlorophyll b has an absorbance peak close to the wavelength of the PC Probe reaction product, hence chlorophyll must be removed from the sample prior to using in the assay (See References 3-4).**
- b. Add 40-50 μ l of sample per well and make up the volume to 100 μ l with ddH₂O. For each sample, prepare parallel sample well(s) to serve as sample background controls.
- c. If desired, Vanillic Acid (a prototypical phenolic acid) may be used as a positive control. Add 50 μ l of the Vanillic Acid 50 mM solution per well into the desired well(s) and make up the volume to 100 μ l with ddH₂O.

