Phenolic Compounds Assay Kit (Colorimetric)

(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.

Phenolic Compounds (phenolic acids, flavonoids, tannins) —*Colorimetric Probe*→ **Diazo Chromophore (λ_{abs} = 480 nm)**

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 µl	Yellow	K527-200-3
Vanillic Acid (50 mM)	500 µl	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:ddH_2O: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 µl of sample per well and make up the volume to 100 µl with ddH_2O. 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 µl of the Vanillic Acid 50 mM solution per well into the desired well(s) and make up the volume to 100 µl with ddH_2O.

Notes:

- Do not use PC Assay Buffer for extraction of phenolic compounds from samples. The buffer should only be used as described in the assay protocol.
- Phenolic content may vary widely between different sample types. For unknown samples, we suggest testing several dilutions to ensure the readings are within the standard curve range.

2. **Standard Curve Preparation:** Dilute the 100 mM Catechin Standard solution at a 1:100 ratio by adding 10 µl of the Standard solution to 990 µl of 70% ethanol (made from 200 proof ethanol and ddH_2O) to obtain a 1 mM Catechin Standard solution. Add 0, 2, 4, 6, 8 and 10 µl of the 1 mM Catechin Standard into a series of wells in a clear 96 well plate to generate 0, 2, 4, 6, 8 and 10 nmol/well of Catechin standard. Adjust the volume of all standard curve wells to 100 µl with ddH_2O.

   Note: If an entire assay plate is being used at one time with numerous samples, it is advisable to prepare the Catechin Standard curve wells after preparing sample wells and their corresponding sample background control wells. The standard curve should be read within 15-20 minutes after addition of all reaction components.

3. **Reaction Mix:**

   a. Add 20 µl of the PC Probe to each of the standard curve and sample reaction wells, **except for the sample background wells**. Add 20 µl of PC Assay Buffer to the sample background wells. Shake the plate to evenly distribute the probe in the wells (while taking care to avoid spillage).

   b. Add 80 µl PC Assay buffer to all of the reaction wells (including standard curve, sample and sample background wells). Shake the plate to ensure adequate mixing of the contents of the wells (while taking care to avoid spillage).

4. **Measurement:** Incubate the plate at room temperature (24-26°C) for 10 minutes with gentle shaking. Measure the absorbance (OD at 480 nm) of all standard curve, sample and sample background control wells in end-point mode.

5. **Calculation:** Subtract the 0 nmol Standard OD_{480} value from all of the standard curve readings and plot the Catechin Standard Curve. If sample background well reading is significant, subtract the sample background control reading from its paired sample reading. Apply the background-corrected sample OD_{480} values to the Catechin Standard curve to get *B* nmol of product (diazo chromophore) generated during the reaction. Use the following calculation to determine mM Catechin Equivalents of the samples:

$$\textbf{Sample Phenolic Compound Concentration (mM Catechin Equivalents)} = \frac{B}{V} \times D = \textbf{nmol/µl} \equiv \textbf{mM Catechin Equivalents}$$

Where: ***B*** is the amount of Diazo Chromophore, calculated from the standard curve (in nmol of catechin)
D is the sample dilution factor (if applicable, *D*=1 for undiluted samples)
V is the volume of sample added to the reaction well (in µl)

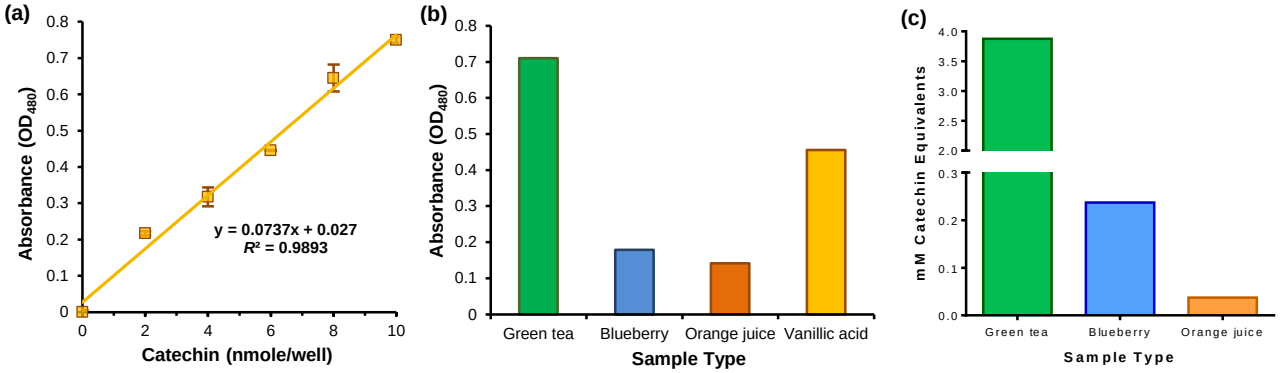

Figure: (a) Catechin Standard curve. **(b)** Absorbance readings for 50 µl diluted solutions of green tea (brewed for 5 min and diluted 1:20 fold with ddH_2O), blueberry methanolic extract (extract made from 50 mg of freeze-dried blueberries in 5 ml of MeOH/ddH_2O/HCl extraction solvent and diluted 1:5 fold with ddH_2O), orange juice (centrifuged to remove pulp and supernatant used without dilution) and 50 µl positive control (vanillic acid 50 mM solution). **(c)** Catechin equivalents (in mM) of green tea, blueberries and orange juice. Catechin equivalency is defined as nmoles of phenolic compounds per µl of solution, equivalent to nmoles of catechin per µl of solution, as calculated from the Catechin Standard curve. Assays were performed following the kit protocol.

VIII. RELATED PRODUCTS:

Total Antioxidant Capacity (TAC) Colorimetric Assay Kit (K274)	Hydrogen Peroxide Assay Kit (K265)
Ferric Reducing Antioxidant Power Assay Kit (K515)	Reactive Oxygen Species (ROS) Detection Assay Kit (K936)

IX. References:

1. Stalikas CD (2007). Extraction, separation, and detection methods for phenolic acids and flavonoids. *J Sep Sci.* **30:** 3268–95.
2. Santos-Buelga C, Gonzalez-Manzano S, Dueñas M, Gonzalez-Paramas AM (2012). Extraction and isolation of phenolic compounds. *Methods Mol Biol.* **864:** 427–64.
3. Lanfer-Marquez UM, Barros RMC, Sinnecker P (2005). Antioxidant activity of chlorophylls and their derivatives. *Food Res Int.* **38:** 885–891.
4. Saowapa R, Soottawat B, Thummanoon P (2015). Extraction, antioxidative, and antimicrobial activities of brown seaweed extracts, Turbinaria ornate and Sargassum polycystum, grown in Thailand. *Int. Aquat. Res*. **7:** 1–16.

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