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Product Information

Phenolic Compounds Assay Kit

Catalog Number **MAK365**
Storage Temperature –20 °C

TECHNICAL BULLETIN

Product Description

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.

The 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 protocol for measuring phenolic compounds, this 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.

The kit is suitable for the measurement of phenolic compounds in fruits, vegetables, beverages (e.g. tea, wine, coffee), food products, plant extracts, and natural or herbal products.

Components

The kit is sufficient for 200 colorimetric assays in 96 well plates.

Component	Quantity
PC Assay Buffer Catalog Number MAK365A	25 mL
PC Probe Catalog Number MAK365B	4 mL
Catechin Standard (100 mM) Catalog Number MAK365C	100 μL
Vanillic Acid (50 mM) Catalog Number MAK365D	500 μL

Reagents and Equipment Required but Not Provided.

- Pipetting devices and accessories (e.g., multichannel pipettor)
- Spectrophotometric multiwell plate reader
- Clear flat-bottom 96 well plates
- Ethanol, 200 proof (Catalog Number E7023)
- Organic solvents (e.g. methanol, acetone) and dilute hydrochloric acid (1 M HCl) for sample extraction

Precautions and Disclaimer

For R&D use only. Not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

The kit is shipped on wet ice. Store components at –20 °C, protected from light. Briefly centrifuge small vials prior to opening.

Preparation Instructions

Reagent Preparation

PC Assay Buffer: Warm to room temperature and vortex prior to use.

PC Probe: Warm to room temperature prior to use.

Catechin Standard (100 mM) and Vanillic Acid (50 mM): Aliquot and store at –20 °C, protected from light. Ensure cap is tightly closed when not in use.

70% Ethanol: Prepare 1 mL by adding 700 μL of 200 proof Ethanol to 300 μL of ultrapure water, mix well.

Procedure

Sample Preparation

1. A variety of fruit, vegetable, and plant samples, beverages, as well as herbal and 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 solution of methanol:ultrapure water:1 M HCl), acid/ethanol or acetone extraction methods. Users may utilize 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. **Do not use** PC Assay Buffer for extraction of phenolic compounds from samples. Phenolic content may vary widely between different sample types. For unknown samples, test several dilutions to ensure the readings are within the standard curve range. Various phenolic extraction methods have been published.[1,2] Fruit or 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).
2. Add 40-50 μL of sample per well and bring the volume to 100 μL with ultrapure water.
3. For each sample, prepare parallel sample well(s) to serve as sample background controls.
4. 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 bring the volume to 100 μL with ultrapure water.

Standard Curve Preparation

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.

Prepare a 1 mM Catechin Standard solution by adding 10 μL of 100 mM Catechin Standard solution to 990 μL of 70% ethanol. Prepare Catechin Standards in desired wells of a clear flat-bottom 96 well plate according to Table 1.

Table 1.
Preparation of Catechin Standards

Well	1 mM Premix	Ultrapure Water	Catechin (nmol/well)
1	0 μL	100 μL	0
2	2 μL	98 μL	2
3	4 μL	96 μL	4
4	6 μL	94 μL	6
5	8 μL	92 μL	8
6	10 μL	90 μL	10

Reaction Mix

1. Add 20 μL of PC Probe to each of the standard curve and sample reaction wells, except for the sample background wells.
2. Add 20 μL of PC Assay Buffer to the sample background wells.
3. Gently shake the plate to evenly distribute the probe in the wells (while taking care to avoid spillage).
4. Add 80 μL of PC Assay buffer to all reaction wells (including standard curve, sample, and sample background wells).
5. Gently shake the plate to ensure adequate mixing of the contents of the wells (while taking care to avoid spillage).

Measurement

Incubate the plate at room temperature for 10 minutes with gentle shaking. Measure the absorbance at 480 nm (A_{480}) of all standard curve, sample, and sample background control wells in end-point mode.

Results

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

   Sample Phenolic Compound Concentration (mM or nmol/μL Catechin Equivalents) =

$$(B/V) \times D$$

where:
B = the amount of Diazo Chromophore, calculated from the standard curve (in nmol of catechin)
V = the volume of sample added to the reaction well
D= Sample dilution factor

Figure 1.
Typical Catechin Standard Curve

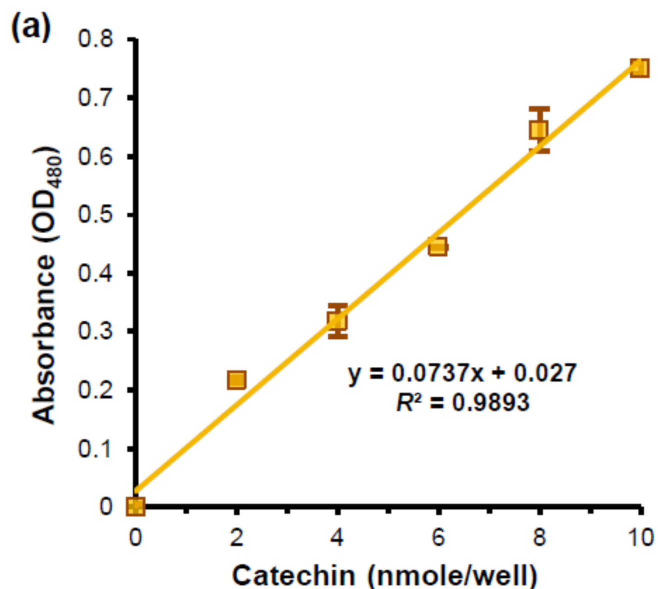
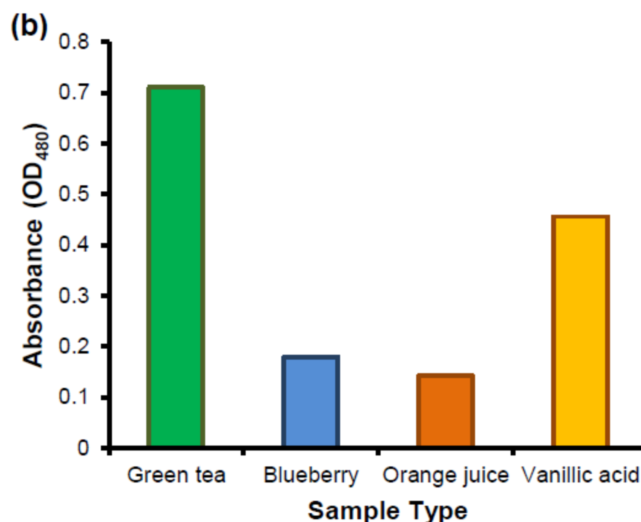
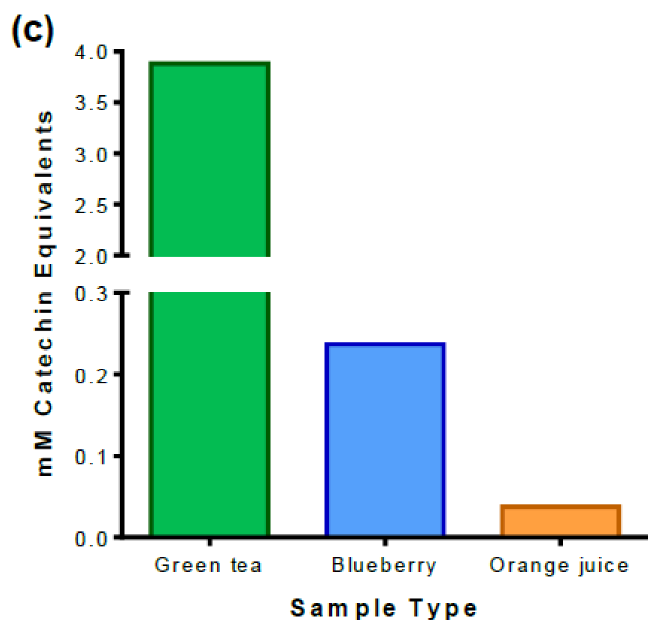


Figure 2.
Typical Absorbance Readings



Absorbance readings for 50 μL of diluted solutions of green tea (brewed for 5 minutes and diluted 20-fold with ultrapure water), blueberry methanolic extract (extract made from 50 mg of freeze-dried blueberries in 5 mL of MeOH/ultrapure water/HCl extraction solvent and diluted 5-fold with ultrapure water), orange juice (centrifuged to remove pulp and supernatant used without dilution) and 50 μL of positive control (vanillic acid 50 mM solution).

Figure 3.
Typical Catechin Equivalents



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 procedure.

References

1. Stalikas, C.D., Extraction, separation, and detection methods for phenolic acids and flavonoids. J. Sep. Sci., **30**, 3268–95 (2007).
2. Santos-Buelga, C. et al., Extraction and isolation of phenolic compounds. Methods Mol. Biol., **864**, 427–64 (2012).
3. Lanfer-Marquez, U.M. et al., Antioxidant activity of chlorophylls and their derivatives. Food Res. Int., **38**, 885–891 (2005).
4. Saowapa, R. et al., Extraction, antioxidative, and antimicrobial activities of brown seaweed extracts, Turbinaria ornate and Sargassum polycystum, grown in Thailand. Int. Aquat. Res., **7**, 1–16 (2015).

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