Version 3a, Last updated 13 June 2025

ab273293
Phenolic Compounds Assay Kit (Colorimetric)

View Kit datasheet: https://www.abcam.com/ab273293
(use https://www.abcam.cn/ab273293 for china, or
https://www.abcam.co.jp/ab273293 for Japan)

For the measurement of Phenolic Compounds in various biological samples.

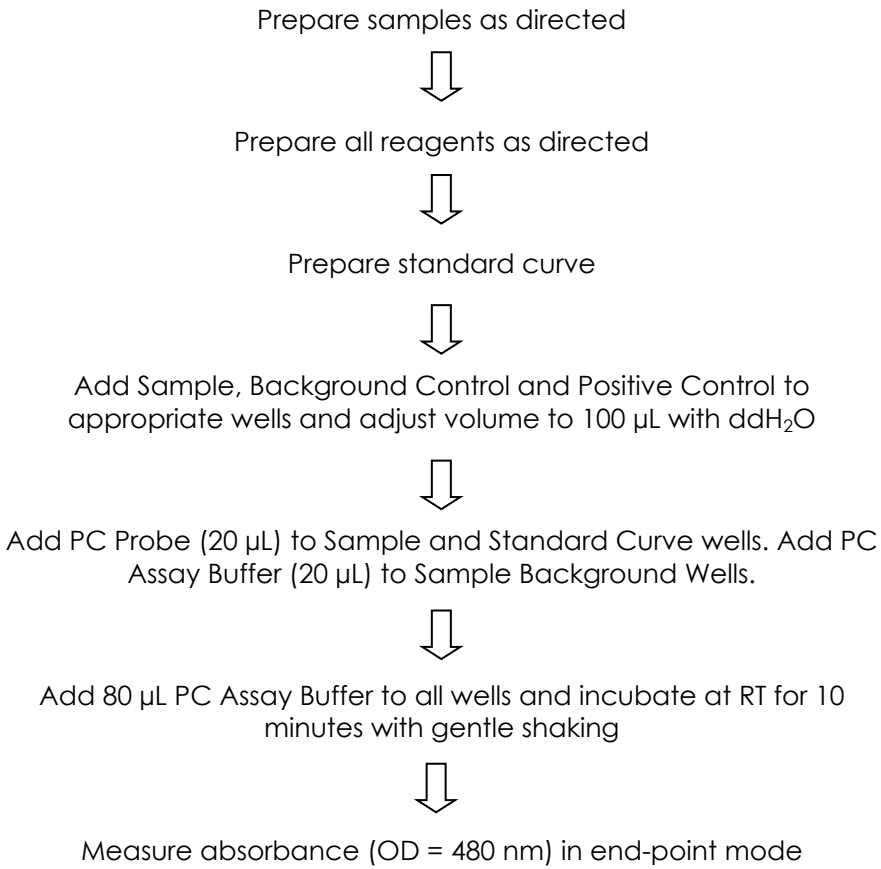
This product is for research use only and is not intended for diagnostic use.

Table of Contents

1. Overview

Phenolic Compounds Assay Kit (Colorimetric) (ab273293) 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.

2. Protocol Summary



3. Precautions

Please read these instructions carefully prior to beginning the assay.

- All kit components have been formulated and quality control tested to function successfully as a kit.
- We understand that, occasionally, experimental protocols might need to be modified to meet unique experimental circumstances. However, we cannot guarantee the performance of the product outside the conditions detailed in this protocol booklet.
- Reagents should be treated as possible mutagens and should be handled with care and disposed of properly. Please review the Safety Datasheet (SDS) provided with the product for information on the specific components.
- Observe good laboratory practices. Gloves, lab coat, and protective eyewear should always be worn. Never pipette by mouth. Do not eat, drink or smoke in the laboratory areas.
- All biological materials should be treated as potentially hazardous and handled as such. They should be disposed of in accordance with established safety procedures.

4. Storage and Stability

Store kit at -20°C in the dark immediately upon receipt.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in the Materials Supplied section.

Aliquot components in working volumes before storing at the recommended temperature.

5. Limitations

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

6. Materials Supplied

Item	Quantity	Storage temperature (before prep)	Storage temperature (after prep)
PC Assay Buffer	25 mL	-20°C	-20°C
PC Probe	4 mL	-20°C	-20°C
Catechin Standard	100 µL	-20°C	-20°C
Vanillic Acid	500 µL	-20°C	-20°C

7. Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully perform this assay:

- Multi-well spectrophotometer capable of measuring OD at 480 nm
- Clear 96-well plate with flat bottom
- Reagent-grade (200 proof) ethanol
- Organic solvents (e.g. methanol, acetone) and dilute hydrochloric acid (1N HCl), for sample extraction

8. Technical Hints

- **This kit is sold based on number of tests. A "test" simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.**
- Selected components in this kit are supplied in surplus amount to account for additional dilutions, evaporation, or instrumentation settings where higher volumes are required. They should be disposed of in accordance with established safety procedures.
- Avoid foaming or bubbles when mixing or reconstituting components.
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions.
- Ensure plates are properly sealed or covered during incubation steps.
- Ensure all reagents and solutions are at the appropriate temperature before starting the assay.
- Samples generating values that are greater than the most concentrated standard should be further diluted in the appropriate sample dilution buffer.
- Make sure all necessary equipment is switched on and set at the appropriate temperature.

9. Reagent Preparation

Briefly centrifuge small vials at low speed prior to opening.

9.1 **PC Assay Buffer:**
Ready to use as supplied. Store at -20 °C. Bring to room temperature and vortex well before use.

9.2 **PC Probe:**
Ready to use as supplied. Warm to room temperature prior to use. Store at –20°C, protected from light.

9.3 **Catechin Standard:**
Aliquot and store at -20°C, protected from light. Close cap tightly. Use within two months.

9.4 **Vanillic Acid:**
Aliquot and store at -20°C. Close cap tightly, protected from light. Use within two months.

10. Sample Preparation

Δ Note: A variety of fruit, vegetable and plant samples, beverages as well as herbal/natural products can be analyzed with this assay.

10.1 Fruit, vegetable and plant extractions:

10.1.1 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.

10.1.2 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.

10.2 Fruit/vegetable juices, liquid herbal products, freeze-dried fruits solubilized in suitable solvents and beverages:

10.2.1 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.

10.2.2 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.

Δ Note: 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.

Δ Note: Phenolic content may vary widely between different sample types.

11. Standard Curve

11.1 Prepare a 1 mM solution of Catechin Standard by diluting 10 µL of Catechin Standard with 990 µL of 70% ethanol (made from 200 proof ethanol and ddH_2O).

11.2 Mix well.

11.3 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 obtain 0, 2, 4, 6, 8 and 10 nmol/well.

11.4 Adjust the volume of each well to 100 µL with ddH_2O.

Standard #	Catechin Standard (µL)	ddH_2O (µL)	Catechin Standard (nmol/well)
1	10	90	10
2	8	92	8
3	6	94	6
4	4	96	4
5	2	98	2
6	0	100	0

Δ 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.

12. Assay Procedure

Thaw all reagents thoroughly and mix gently.

Δ Note: Phenolic content may vary widely between different sample type. For unknown samples, we suggest testing dilutions to ensure the readings are within the Standard Curve range.

12.1.1 For Sample (S), prepare two wells for each sample labeled "Sample Background Control" (BC), and "Sample" (S). Add 40 -50 µL sample into each of these wells.

12.1.2 For Positive Control, Vanillic Acid (a prototypical phenolic acid) may be used. Add 50 µL of the Vanillic Acid 50 mM solution.

12.1.3 Adjust the volume of Sample, Sample Background Control and Positive Control to 100 µL/well with ddH_2O. Mix well.

12.1.4 Add 20 µL of the PC Probe to each of the standard curve and sample reaction wells, **except for the sample background wells**.

12.1.5 Add 20 µL of PC Assay Buffer to the sample background wells.

12.1.6 Shake the plate to evenly distribute the probe in the wells (while taking care to avoid spillage).

12.1.7 Add 80 µL PC Assay buffer to all of the reaction wells (including standard curve, sample and sample background wells).

12.1.8 Shake the plate to ensure adequate mixing of the contents of the wells (while taking care to avoid spillage).

12.1.9 Incubate the plate at room temperature (24-26°C) for 10 minutes with gentle shaking.

12.1.10 Measure the absorbance (OD at 480 nm) of all standard curve, sample and sample background control wells in end-point mode.

13. Calculations

13.1 Subtract the 0 nmol Standard OD480 value from all of the standard curve readings and plot the Catechin Standard Curve.

13.2 If sample background well reading is significant, subtract the sample background control reading from its paired sample reading.

13.3 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.

13.4 Use the following calculation to determine mM Catechin Equivalents of the samples:

$$Sample\ Phenolic\ Compound\ Concentration = \frac{B}{V} x\ D = \frac{nmol}{\mu L} = \ mM\ Catechin\ Equivalents$$

B = the amount of Diazo Chromophore, calculated from the standard curve (in nmol of catechin)
D = the sample dilution factor (if applicable, D=1 for undiluted samples)
V = the sample volume added into the reaction well (µL).

14. Typical Data

Typical standard curve – data provided **for demonstration purposes only**. A new standard curve must be generated for each assay performed.

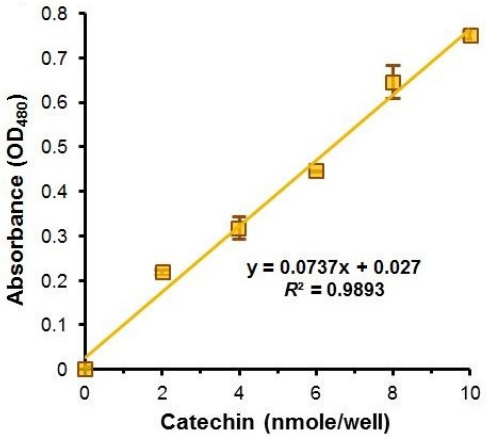


Figure 1. Catechin standard curve.

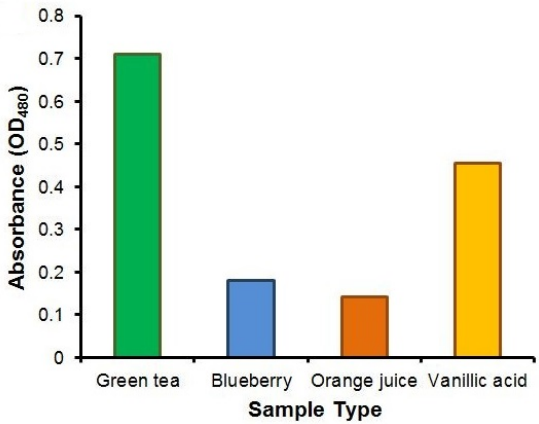


Figure 2. Absorbance readings for 50 µL diluted solutions of green tea (brewed for 5 minutes and diluted 1:20 fold with ddH_2O), blueberry methanolic extract (extract made from 50 mg of freeze-dried blueberries in 5 mL of MeOH/ddH_2O/HCl extraction solvent and diluted 1:5 fold with ddH_2O), orange juice (centrifuged to remove pulp and supernatant used without dilution) and 50 µL positive control (vanillic acid 50 mM solution).

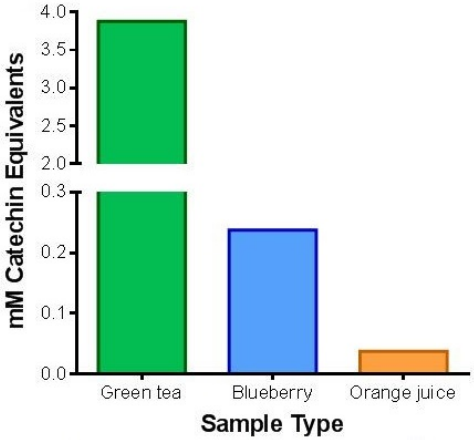


Figure 3. Catechin equivalents (in mM) of green tea, blueberries and orange juice. Catechin equivalency is defined as nmoles of phenolic compounds per µl of solution, equivalent to nmoles of catechin per µl of solution, as calculated from the Catechin Standard curve. Assays were performed following the kit protocol.

15. FAQ / Troubleshooting

General troubleshooting points are found at www.abcam.com/assaykitguidelines.

16. Notes

Technical Support

For all technical or commercial enquiries please go to:

www.abcam.com/contactus

www.abcam.cn/contactus (China)

www.abcam.co.jp/contactus (Japan)