Determination of Total Polyphenol Content in Green Tea Extract by Folin–Ciocalteu Assay

Microplate Reader Application | MR-1000S

Application

Quantitative determination of total polyphenol content (TPC) in green tea extract using the Folin–Ciocalteu colorimetric assay. The assay is measured at 765 nm and quantified against a gallic acid calibration curve, with results expressed as gallic acid equivalents (GAE).

Principle

Phenolic compounds react with Folin–Ciocalteu reagent in the presence of sodium carbonate to form a blue-colored complex. The color intensity increases with the amount of reactive phenolic compounds. Absorbance is measured at 765 nm and compared with gallic acid standards.

Workflow

Green tea extract → Add Folin–Ciocalteu reagent → Add Na_2CO_3 → Incubate in the dark → Read OD765 → Gallic acid standard curve → Calculate TPC as GAE

Instrument Setup

Parameter	Recommended setup
Instrument	MR-1000S Microplate Reader
Measurement mode	Endpoint absorbance
Wavelength	765 nm
Plate	96-well microplate
Replicates	Standards, blank and samples in replicate
Quantitation	Gallic acid calibration curve → unknown concentration → report as GAE

Material and Standards

Item	Published method condition
Green tea extract	Prepared in water at 100 µg/mL
Gallic acid standards	10, 20, 30, 40 and 50 µg/mL
Blank	Water
Folin–Ciocalteu reagent	10% solution
Sodium carbonate	7.5% Na_2CO_3
Item	Published method condition

Microplate Assay Procedure

Step	Volume / condition
1. Add standard, sample or blank	50 µL per well
2. Add 10% Folin–Ciocalteu reagent	50 µL per well
3. Initial reaction	Stand for 3 min
4. Add 7.5% Na_2CO_3	50 µL per well and mix
5. Incubation	60 min at 25°C, in the dark
6. Measurement	Read absorbance at 765 nm

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Result

Based on the measured OD765 values, the microplate reader automatically calculated the concentrations of the unknown samples from the standard curve. Green tea B showed the highest calculated concentration (37.0 μg/mL), followed by Green tea A (25.2 μg/mL) and Beverage C (14.2 μg/mL), demonstrating quantitative determination of total polyphenol content using the gallic acid calibration method.

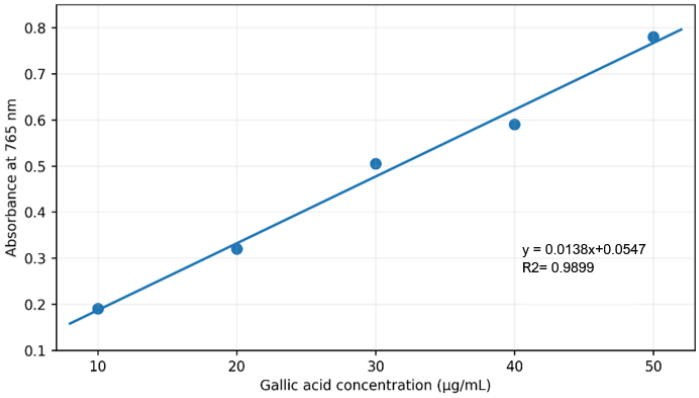

Figure 1: Gallic acid calibration curve

Table 1 Quantitative determination of total polyphenol content in unknown samples using the gallic acid calibration curve

Sample	OD765	GAE Concentration
Green tea A	0.402	25.2 μg/mL
Green tea B	0.565	37.0 μg/mL
Beverage C	0.250	14.2 μg/mL

Conclusion

The MR-1000S enables quantitative total polyphenol analysis by measuring absorbance at 765 nm, generating a gallic acid calibration curve, and automatically calculating the concentrations of unknown samples. Results can be expressed as gallic acid equivalents (GAE), providing a convenient solution for routine food and beverage quality control.

Reference

AOAC Official Method 2017.13. Total Phenolic Content in Extracts: Folin-C (Folin and Ciocalteu) Colorimetric Method.
Comparison of colorimetric methods for the analysis of total polyphenols in green tea extracts. Bioscience, Biotechnology, and Biochemistry. 2024;88(7):798–805.
Determination of Total Phenolic Content Using the Folin-C Assay: Single-Laboratory Validation, First Action 2017.13. Journal of AOAC INTERNATIONAL. 2018;101(5):1466–1472.