Polyphenols (Folin Ciocalteu) Assay

(Cat. # BAQ056)

INTRODUCTION

Polyphenols have become an intense focus of research interest because of their perceived health-beneficial effects. They occur in a variety of fruits, vegetables, nuts, seeds, flowers, bark, beverages, and even some manufactured food, as a component of the natural ingredients used. They have been reported to exhibit anti-carcinogenic, anti-atherogenic, anti-ulcer, anti-thrombotic, anti-inflammatory, immunomodulating, anti-microbial, vasodilatory and analgesic effects.

Interest in the research of polyphenols from different natural sources has grown because polyphenols can be utilized as antioxidants in the food industry, and they benefit human health in various ways. The beneficial effects of polyphenols on human health could be due to their free radical scavenger properties, blocking the deleterious action of these molecules on cells.

This assay is based on Folin-Ciocalteu method. The FC reagent contains phosphomolybdic/ phosphotungstic acid complexes. The method relies on the transfer of electrons in alkaline medium from phenolic compounds to form a blue chromophore constituted by a phosphotungstic/phosphomolybdenum complex where the maximum absorption depends on the concentration of phenolic compounds. The reduced Folin-Ciocalteu reagent is detectable with a spectrophotometer in the range of 690 to 710 nm. The reaction temperature has been used to reduce the time necessary to attain the maximum color (T= 37°C). Generally, gallic acid is used as the reference standard compound and results are expressed as gallic acid equivalents (mg/mL).

The assay has been used as a measure of total phenolics in natural products, but the basic mechanism is an oxidation/reduction reaction. In the original Folin-Ciocalteu assay, the carbonate buffer is used for pH adjustment and the end-point of the reaction was attained after 120 min at room temperature, which makes its implementation for routine analysis difficult. The proposed method was performed in a 96-well microplate format and it was applied to several phenolic compounds and food products (wines, beers, infusions, juices).

ITEM(S) SUPPLIED

Description	Size
FC Reagent A	4 vials
FC Reagent B*	2 bottles
FC Reagent C	1 bottle
Standard	4 vials

** If FC Reagent B is a gel-like consistency then warm until it returns to a homogenous liquid. Heat to a maximum of 55°C.*

STORAGE CONDITIONS

This kit is shipped at ambient temperature. Store all the reagents as indicated on the labels. If stored and used as directed this kit is stable for 12 months.

ADDITIONAL ITEMS REQUIRED

- Spectrophotometer microplate reader that can measure 700 nm
- 96 well microtiter plate for microplate assay.
- 1.5ml Tubes
- Distilled or deionized water

PREPARATION BEFORE USE

1. Dilute the FC Reagent A in a relation 1: 10 with distilled water in a vial (not included). This dilution will be called RA Working Solution.
2. Add 1.5 mL of FC Reagent C in each Standard vial. Once dissolved, keep it at -20°C.
3. Dilute the unknown samples until they reach an absorbance within the limits of the standard curve (See below for absorbance values).

PROTOCOL

1. Prepare standards containing a range of 0 to 300 µg/mL in a volume of 200 µL.
2. Pipette 20 µL of each standard and unknown samples into a microplate well. Refer to the Table 1 as a guide for diluting the standard. For the diluent, use the same buffer as in the samples.
3. To each well, add 100 µL of RA Working Solution.
4. Add 80 µL of Reagent B to each well.

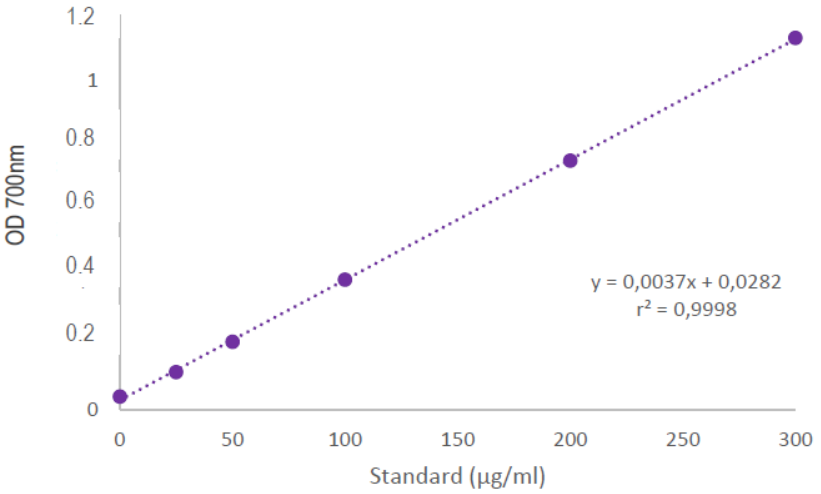
Table 1. Preparation of diluted standards

Sample	Standard [µL]	Diluent [µL]	Gallic acid [µg/mL]
S1 (Blank)	---	200	---
S2	5	195	25
S3	10	190	50
S4	20	180	100
S5	40	160	200
S6	60	140	300

5. Measure the absorbance of these standards, blanks and unknown samples at 700 nm (T= 37°C).

DATA ANALYSIS

1. If the spectrophotometer or microplate reader was not zeroed with the blank, then subtract the average blank value from the standard and unknown sample values.
2. Create a standard curve by plotting A700 nm (y-axis) vs. standard, µg (x-axis). Determine the unknown sample concentration using the standard curve.
3. Standard curve example for microplate assay procedure is shown:



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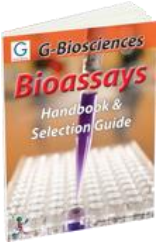

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