

Total Phenol Folin-Ciocalteu Procedure Updated

Product #: PHEN-fc F60 (30 Tests)

Photometric, micro-Method

PHEN-fc F150 (75 Tests)

INTENDED USE

Total Phenol PHEN-fc FLEX-REAGENT™ is the traditional Folin-Ciocalteu method intended for determination of total phenols and polyphenols in liquid samples. This reagent has broad reactivity for phenolic compounds, including tannins; refer to Note 1 below to correct for interfering substances which may affect the accuracy of wine phenolic results.

REAGENT	Quantity/Kit
	30T 75T
1. F-C Reagent	5mL 13mL
2. Carbonate Solution	9mL 23mL
3. Gallic Acid Standard 2000 mg/L	1mL 1mL

A 4-Level Standards Kit is available from Unitech.

SAMPLES

Turbid samples should be filtered. Fermentation samples may be clarified by centrifugation.

REAGENTS & STORAGE

Components as supplied are ready to use and stable through the labeled expiration date when stored at 2-8 oC. System parameters: Wavelength 750 - 760nm, Absorbance Range 0-2.5A, pathlength 1.0 cm.

MANUAL PROCEDURE (refer to **Notes** for White Wine)

Prepare sufficient F-C Working Reagent (WRgt.) as shown:

	1 Test	6 Tests	12 Tests	25 Tests
F-C Reagent (#1)	0.20mL	1.0mL	2.0mL	4.0mL
Deionized Water	2.0mL	10 mL	20 mL	40 mL
WRgt (Approx.Total)	2 mL	11 mL	22 mL	44 mL

Warm WRgt and Carbonate Solution room temperature.

1. Pipet water into the Reagent Blank cuvette and pipet standards and samples into reaction cuvettes.

High Phenol Range, e.g. Red Wine samples

Pipette into Cuvettes	Reagent Blank Cuvette	Reaction Cuvettes
Sample & Standard		15µL
DI water	15µL	
F-C W.Rgt.	1.70 mL	
Mix, wait 3 minutes		
Carbonate Solution	300 uL	
Mix, wait 2 hours at room temp. (or 30 min at 37C) read Absorbances (A ₇₆₀).		

2. Add F-C Working Rgt., mix & incubate, then add Carbonate Solution, mix & incubate - as shown on the table.

3. Zero spectrophotometer with Reagent Blank. Read absorbance values.

This procedure is linear in the range 250 – 4000 mg/L. Dilute over-range samples with deionized water and reassay. (Refer to LINEARITY section for low-range protocol, i.e. white wine.)

CALCULATIONS

Single Standard - Total Phenol concentration is proportional to ABS. Calculate mg/L values from the ABS obtained for the Standard, as follows. Correct or any sample dilutions (DF = dilution factor.)

Total Phenol mg/L = 2000 x $\frac{A}{A_{\text{standard}}} \times DF$ where DF is the dilution factor

Multi-Point Standard Method - Run a 4-point standard curve. Plot ABS Values vs. concentration for each standard. Calculate mg/L of each sample from this curve. A 4-Level Gallic Acid Standard kit is available from Unitech Scientific.

ChemWell Procedure

Place F-C Reagent, Carbonate Solution and Standard(s) in reagent rack per Loading Instructions. Contact Unitech Scientific for assay procedure and technical support.

SIGNIFICANCE OF MEASUREMENTS

Phenolics in wine include tannins, anthocyanins, polymeric pigments and monomeric phenols (e.g. caffeic acid, caftaric acid, catechins, quercetin, kaempferol, and gallic acid.) They are responsible for wine color, bitterness, and astringency. This Folin-Ciocalteu method has broad reactivity to phenols (refer to Note 1 below), and is sensitive and relatively independent of the degree of polymerization (e.g. mono-, di- or trimer).

Color and flavor profiles of finished wine are affected by grape selection and winemaking techniques. Total phenol content, as well as color and hue data, can provide valuable information for optimizing these processes.

QUALITY CONTROL

For quality control, the phenol concentration obtained for a white wine sample may be compared with the result of that wine spiked with gallic acid. Use the difference in Phenol values to calculate the "% Expected" recovery of gallic acid spiked.

Procedural factors that may affect the performance of this test include proper instrument function, temperature standard, glassware cleanliness, and pipetting accuracy.

Photometrically (750–770nm) measured reaction products are the basis for Total Phenol determination; results are expressed in Gallic Acid equivalents.

NOTES

1. Interfering Substances: Structures containing phenolic rings (e.g. sugars and ascorbic acid in wine, proteins, nucleic acids) and others react with F-C reagent. Correct results for interference.

- o Reducing sugars, e.g. glucose & fructose – expect minor interference; comparison of uncorrected total phenol values for samples of similar brix values may be of utility in some winemaking applications.
- o Sulfites (significant for white wine with sulfites >50mg/L)
- o Ascorbic acid - expect a relative mass response of 0.68; e.g. for a wine containing 30mg/L ascorbate, subtract 20.4mg/L from the total phenol value.
- o Samples with protein, nucleic acids, and other phenolic compounds may exhibit large positive errors.

2. Low Phenol Range, White Wine:

Phenol may be measured from 50 – 400 mG/L using the following protocol.

Pipette into Cuvettes	Reagent Blank Cuvette	Reaction Cuvettes
Sample & Standard		150µL
DI water	150µL	
F-C W.Rgt.	1.55 mL	
Mix, wait 3 minutes		
Carbonate Solution	300 uL	
Mix, wait 2 hours at room temp. (or 30 min at 37C) read Absorbance (A ₇₆₀).		

Standardize this assay using a 250 mG/L standard; prepare sufficient standard for each assay - mix 50µL of 2000mG/L Standard plus 350µL DI Water.

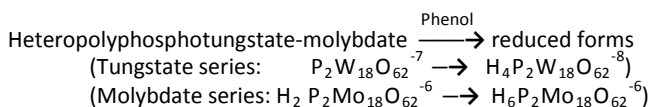
Calculation, White Wine (i.e. Low Range Protocol)

Total Phenol mg/L = 250 x $\frac{A}{A_{\text{standard}}}$ x DF where DF is the dilution factor

METHODOLOGY & CHEMICAL PRINCIPLES

This reagent, based on the Slinkard and Singleton method and the early work of Singleton and Rossi is a colorimetric oxidation/reduction method for phenolic compounds.

Phenols stoichiometrically reduce phosphomolybdic/phosphotungstic acid:



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