

### Total Phenol

Photometric, micro-Method

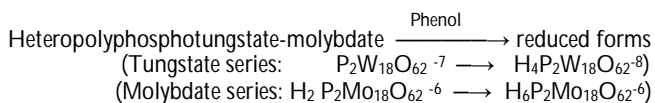
Product #: PHEN-F60 (30 Tests), PHEN-F150 (75 Tests)  
PHEN-F500 (250 Tests)

#### INTENDED USE

Total Phenol FLEX-REAGENT™ is intended for determination of total phenols and polyphenols in wine, tea and other non-protein liquid samples.

#### METHODOLOGY & CHEMICAL PRINCIPLES

This reagent, based on the Slinkard and Singleton method<sup>1</sup> and the early work of Singleton and Rossi<sup>2</sup> is a colorimetric oxidation/reduction method for phenolic compounds. This Folin-Ciocalteu method is sensitive, quantitative and relatively independent of the degree of polymerization (e.g. mono-, di- or trimer), and is generally preferred over other analyses of wine phenols<sup>3</sup>. Proteins, nucleic acids and ascorbic acid interfere, correction may be required. Phenols stoichiometrically reduce phosphomolybdic/phosphotungstic acid:



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

#### PHEN FLEX-REAGENTS

Concentration	Quantity/Kit	as Formulated	30T	75T	250T
1. <u>F-C Reagent</u>			5mL	13mL	40mL
Li Sulfate	15%				
Na Tungstate 2 H <sub>2</sub> O	10%				
HCl	≥2.5%				
H <sub>2</sub> PO <sub>3</sub>	4.2%				
Molybdic Acid Na 2 H <sub>2</sub> O	2.5%				

2. <u>Carbonate Solution</u>		9mL	23mL	75mL
Sodium Carbonate	80% sat. soln.			
3. <u>Gallic Acid Standard</u>	2000 mg/L	1mL	1mL	5mL

A 4-Level Total Phenol Standards Kit is available from Unitech.

#### REAGENTS & STORAGE

Components as supplied are ready to use and stable through the labeled expiration date when stored at 2-8 °C.

#### ChemWell PROCEDURE

Place F-C Reagent, Carbonate Solution and Standard(s) in reagent rack per Loading Instructions.

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

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
<b>WRgt (Approx.Total)</b>	<b>2 mL</b>	<b>11 mL</b>	<b>22 mL</b>	<b>44 mL</b>

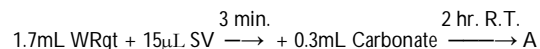
Allow WRgt and Carbonate Solution to reach room temp.

- Pipet water into the Reagent Blank cuvette and pipet standards and samples into reaction cuvettes.
- Add F-C Working Rgt., mix & incubate, then add Carbonate Solution, mix & incubate - as shown on the following table. (Refer to LINEARITY section for low-range protocol, i.e. white wine)
- Zero spectrophotometer with DI water. Read absorbance values.

#### High Phenol Range, e.g. Red Wine samples

Pipette into Cuvettes	Reagent Blank Cuvette	Reaction Cuvettes
Sample		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 <sub>760</sub> ).		

System parameters: Wavelength 750 - 760nm, Absorbance Range 0-2.5A, pathlength 1.0 cm., the schematic protocol is:



#### CALCULATIONS

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

$$\text{High Range Protocol (e.g. red wine)} \\ \text{Total Phenol mg/L} = 2000 \times \frac{A_{\text{PHEN}} \times DF}{A_{\text{PHEN(standard)}}$$

$$\text{Low Range Protocol (e.g. white wine)} \\ \text{Total Phenol mg/L} = 200 \times \frac{A_{\text{PHEN}} \times DF}{A_{\text{PHEN(standard)}}$$

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. 4-Level Gallic Acid Standards are available from Unitech Scientific.

#### SAMPLES

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

## ACCURACY

This method has been widely used for wine evaluation for 30 years, and is the AOAC method for determining total polyphenol content of wine. Potential interferents include:

1. 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.
2. Sulfites (significant for white wine with sulfites >50mg/L)
3. 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.
4. Samples with protein, nucleic acids, and other phenolic compounds may exhibit large positive errors.

Refer to Reference 3, below for details of method specificity.

## SIGNIFICANCE OF MEASUREMENTS

Phenolics in wine include tannins, anthocyanins, polymeric pigments<sup>4</sup> and monomeric phenols (e.g. caffeic acid, caftaric acid, catechins, quercetin, kaempferol, and gallic acid<sup>5</sup>) and are responsible for wine color, bitterness, and astringency. Color and flavor profiles of finished wine are affected by grape selection and winemaking techniques<sup>6</sup>. 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 obtained for a white wine sample may be compared with that wine spiked with gallic acid. Use the difference in Phenol values for these samples to calculate the “% Expected” recovery of gallic acid spiked. Factors that may affect the performance of this test include proper instrument function, temperature standard, glassware cleanliness, and pipetting accuracy.

## LINEARITY

Assay parameters above permit measurement of Total Phenol in the range 200 – 4000 mG/L. Dilute over-range samples with deionized water and reassay.

## NOTES

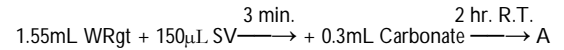
Phenol may be measured from 20 – 400 mG/L using the following protocol. Standardize this assay using a 200 mG/L standard; prepare sufficient standard for each assay - mix 20µL of the Standard supplied (2000 mG/L) with 180µL DI Water.

### Low Phenol Range, e.g. White Wine samples

Pipette into Cuvettes	Reagent Blank Cuvette	Reaction Cuvettes
Sample		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 <sub>760</sub> ).		

System parameters: Wavelength 750–770 nm, Absorbance Range 0-2.5A, pathlength 1.0 cm.

The schematic protocol is:



## REFERENCES

1. Slinkard, K; Singleton, VL, Total Phenol Analysis: Automation and Comparison with Manual Methods. AJEV (1977), 28: 49-55.
2. Singleton, VL, Rossi. J.A., Jr., AJEV (1965) 16, 144.
3. Chemistry of Winemaking, Advances in Chemistry Series 137, A. Dinsmoor Webb. American Chemical Society, 185 ff (1974.)
4. C.A. Sims, K.R.Morris, Effects of Acetaldehyde and Tannins on the Color and Chemical Age of Red Muscadine (Vitis rotundifolia) Wine. JEV 37(2):163-65, 1986.
5. Hames F. Harbertson, D.DeBeer, A.L.Waterhouse, and D.O. Adams, Use of Ferric Chloride for the Measurement of Total Phenolics in Red and White Wine. AJEV 55:3 (2004) ABSTRACT
6. Concepts in Wine Chemistry, Yair Margalit. Wine Appreciation Guild, 43-4 and 126-7 (2004)

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