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FLEX-REAGENT™

Total-Polyphenols *enz*

New Reagent & Procedure

Product #: P-PHEN*enz* F40 (20 Tests)

Enzymatic, Polyphenol Oxidase *(PPO) enzymatic method for wines & must*

P-PHEN*enz* F120 (60 Tests)

INTENDED USE

This reagent is intended for enzymatic determination of polyphenols in wine; specificity includes polymeric anthocyanins and tannins.

KIT CONTENTS: P-PHEN*enz*	**20-T**	**60-T**
Sample Blank Solution	40 mL	2x60 mL
Chromogen R-1a	29 mL	2x44 mL
Catalyst R-1b	3.5 mL	10 mL
R2 [*PPO* Enzyme]	7.5 mL	21 mL
Standard, 2100mg/L	2 mL	2 mL

REAGENTS

Kit contents are ready to use and stable through the labeled expiration date when stored at room temperature between 2 to 8° C and protected from direct light in a tightly closed bottle.

PREPARATION & STORAGE

Prepare Working Reagent immediately before use. Mix R-1a Chromogen and R-1b Catalyst in the ratio of 9 + 1.

e.g, to analyze a Blank, Standard, and 8 samples:

Chromogen R-1a	18mL
Catalyst R-1b	2mL
TOTAL VOLUME	20mL

PROCEDURE – Red Wine

Measure photometrically at 520nm, 1 cm lightpath. The following red wine method is linear to 3750 mg/L of Gallic Acid equivalents. (Refer also to **NOTE 1 - White Wine** procedure, below.) In this test, the ABS contributed by the sample (measured in a Blank cuvettes) is subtracted from the ABS in each reaction cuvette.

Hence, for the following steps, prepare 2 sets of cuvettes, the Blank & Reaction sets.

1. Two Zero-Rgt Cuvettes will be used to zero the spec. Pipet 30uL DI Water into Zero-Rgt Blank Cuvette & Reaction Cuvette, as shown in the table.
2. Prepare a Blank & Reaction Cuvette for the Standard and each Sample:
   a. Pipet 30uL of Standard into both (BL & Rx) Standard Cuvettes.
   b. Pipet 30*µL* of the 1st Sample into the next pair (Blank & Reaction) of cuvettes.
   c. Repeat step b above for each additional Sample, as shown in the table.
3. Add 2000*µL* of **Sample Blank** Solution into each Blank Cuvette (ref. Steps 1 & 2 above*)* as shown in the table.
4. Add 1650*µL* **Working-Rgt** into each Reaction Cuvette *(ref. Steps 1 & 2 above)* followed by addition of 350uL **R2 [PPO]** (as shown in the Table). Incubate at 37C.
5. Set spectrophotometer to 520nm; zero with *Zero-Rgt* Blank Cuvette and read Standard & Sample Blank cuvettes.
6. Re-zero Spec with *Zero-Rgt* **Reaction Cuvette;** read Standard and Sample Reaction cuvettes.

ADD	**Volume/Cuvette**			
	Zero-Rgt Cuvettes		**Standard(s) or Sample(s)**	
	Blank Cuvette	**Reaction Cuvette**	**Blank Cuvette**	**Reaction Cuvette**
DI Water	30*µL*	30*µL*	-	-
Standard or Sample			30*µL*	30*µL*
Sample Blank	**2000µL**	-	**2000µL**	-
Working-Rgt	-	1650*µL*	-	1650*µL*
R2 [PPO Enz]		350*uL*		350*uL*
Mix, wait 5-minutes at 37C				
Zero Spec & **Read Blank Cuvettes** - *refer to Step #5*				
Zero Spec & **Read Reaction Cuvettes** - *refer to Step #6*				

CALCULATION

Calculate Net ABS values by subtracting corresponding Blank ABS values from Reaction ABS values:

$$\text{Net } A_{STD} = A_{STD} - A_{STD\text{-}BL}$$

$$\text{Net } A_{SAMPLE} = A_{SAMPLE} - A_{SAMPLE\text{-}BL}$$

Calculate the Total Polyphenols concentrations (based on the 2100mg/L standard):

$$\textbf{Polypyhenols, mg/L} = 2100 \times \frac{\text{Net } A_{SAMPLE}}{\text{Net } A_{STD}}$$

Automation PROCEDURE

Prepare sufficient **Working Reagent** for the day's testing, per manual instructions above. Refer to table for volumes require for various CW batch sizes:

# Tests	**15**	**27**	**112**	**194**
Chrom R-1a	3.60	5.40	18	30
Catalyst R-1b	0.40	0.60	2	3.34
Total WRgt Vol	**4.00**	**6.0**	**20.0**	**33**

Place the following in reagent rack, per Loading Instructions: Sample Blank Solution, **Working Reagent**, **R2** [*PPO* Enzyme] and **Standard**. Contact Unitech Scientific for the ChemWell for Wine™ automated test protocol and technical support.

QUALITY CONTROL

Each laboratory should establish its own internal Quality Control scheme and procedures for corrective action if controls do not recover within the acceptable tolerances.

SPECIFICITY & STANDARDIZATION This enzymatic method is specific for polyphenols and has minimal reactivity with monomeric phenols (e.g. malvidin 3 glucoside and other monomeric Anthocyanins do not react with polyphenol oxidase.) Standardized is based on a mixture of polyphenols value assigned by a reference method. (No reference standard adequately represents the complexity of wine polyphenols.) To express polyphenol concentration in alternate units, convert as follows:
Tannic Acid mg/L = Gallic Acid * 1.57
Catechin mg/L = Gallic Acid * 1.24

SIGNIFICANCE OF MEASUREMENTS

Polyphenols represent a diverse class with multiple phenolic ring structures containing an hydroxyl group. Phenolics in wine include tannins, anthocyanins, polymeric pigments and monomeric phenols (e.g. caffeic acid, caftaric acid, catechins, quercetin, kaempherol, and gallic acid.) Polyphenols, responsible for wine color, bitterness, and astringency, are affected by grape selection and winemaking techniques. Total polyphenol & Anthocyanin content, as well as color and hue data, can provide valuable information for optimizing these processes.

METHODOLOGY & CHEMICAL PRINCIPLES

Polyphenols in the wine sample react in the presence of Polyphenol Oxidase [*PPO*] enzyme with the Chromogen producing a color measured at 520nm which is proportional to the total polyphenols concentration.

NOTE 1 - White Wine procedure **- Low Range**

Phenol may be measured from 20 – 400 mG/L using a 10-fold **larger Sample Volume, 300uL** as shown on the table.
Standardize this assay using a 210 mG/L standard:

- prepare sufficient standard prior to each assay by diluting the 2100mg/L Standard provided at 1:10 (e.g. 70uL Standard + 630uL DI Water.)
- Two Zero-Rgt Cuvettes will be used to zero the spec. Label a Zero-Rgt Blank & Reaction Cuvette. Pipet 300uL DI Water into each of these cuvettes, as shown in the table.
- For each Standard and each Sample, label a Blank & Reaction Cuvette. Pipet 300uL each Standard & Sample into both (BL & Rx) sets of cuvettes, as shown in the table.
- Add 2000uL W-BL solutions to each blank cuvette (refer to Table)
- Add 1650 uL W-Rgt to each reaction cuvette, follow by 350uL R2 [PPO] (refer to Table). Incubate at 37C.
- Zero the Spec with **Zero-Rgt Blank Cuvette**; read Standard & Sample Blank cuvettes.
- Re-zero Spec with **Zero Reaction Cuvette;** read Standard and Sample Reaction cuvettes.

Low Range, White Wine Prodedure

ADD	Volume/Cuvette			
	Zero-Rgt Cuvettes		Standard(s) or Sample(s)	
	Blank Cuvette	Reaction Cuvette	Blank Cuvette	Reaction Cuvette
DI Water	300µL	300µL	-	-
Standard or Sample			300µL	300µL
W-BL	2000µL	-	2000µL	-
W-Rgt	-	1650µL	-	1650µL
R2 [PPO Enz]		350uL		350uL
Mix, wait 5-minutes at 37C Zero Spec with Zero-Rgt Blank & **Read Blank Cuvettes** Zero Spec with Zero Reaction Cuvette & **Read Reaction Cuvettes**				

CALCULATION, White Wine

Calculate Net ABS values by subtracting corresponding Blank ABS values from Reaction ABS values:

Net A_{STD} = A_{STD} - A_{STD-BL}
Net A_{SAMPLE} = A_{SAMPLE} - $A_{SAMPLE-BL}$

Calculate the Total Polyphenols concentrations (based on 10-fold diluted 2100mg/L standard):

Polypyhenols, mg/L = 210 X $\frac{\text{Net } A_{SAMPLE}}{\text{Net } A_{STD}}$

Unitech Scientific
12026 Centralia Road Suite H
Hawaiian Gardens, CA 90716
Tel: 562 924-5150 Fax: 562-809-3140
www.unitechscientific.com