

**GLYCEROL** *New Automation Procedure*  
**Enzymatic, UV-Method**

Product # **GLY-40** (20 Tests)  
**GLY-80** (40 Tests)

**INTENDED USE**

This Glycerol Kinase/Glycerol PhosphoOxidase reagent is intended for the determination of Glycerol in wine or fruit juice.

KIT CONTENTS	GLY-40	GLY-80
Blank Solution	50mL	2 X 50mL for Automation only
Reagent #1	40mL	2 X 40mL
Reagent #2 (GPO)	10mL	20mL
Glycerol Std, 0.4G/L*	2mL	2mL

\* Corresponds to 12 G/L in wine; refer to Sample Dilution

**STORAGE & REAGENT PREPARATION**

All reagents are liquid, ready to use and stable until the labeled expiration date when stored refrigerated (2-8°C) in original containers. See APPENDIX Reagent Preparation for Automation.

**SAMPLE PREPARATION**

**Clarification**

Turbid samples should be filtered or centrifuged prior to analysis. Samples with Glycerol results >13G/L should be diluted and reassayed; multiply result by dilution factor.

**Sample Dilution**

Dilute wine samples 1:30 with DI Water prior to assay; use Standard undiluted (as is.)

**PROCEDURE**

**System parameters:** Wavelength 546nm (520 – 550), Absorbance Range 0-2.0A, 1cm pathlength.

Label one cuvette for Standard and each Sample.

1. Pipet 100 uL standard, samples into cuvettes, as shown on the following table, using micropipettes.
2. Add Reagent 1 to each reaction cuvette, mix, and wait, as shown in the Table.
3. Zero the spectrophotometer using D.I. water, read Initial ABS.

Pipet into ...	Standard Cuvette	Sample Cuvettes
Standard	100 µL	
Sample		100 µL
Reagent #1	2 mL	
Mix and wait 2 minute Read (ABS <sub>INITIAL</sub> ) of each cuvette		
Reagent #2	500 µL	
Mix and wait 10 minutes Read ABS <sub>FINAL</sub> of each Cuvette		

4. Dispense Reagent 2 into each reaction cuvette, Mix, wait, and read final ABS.

**CALCULATION**

Calculate the Glycerol concentration:

**Glycerol G/L =**  

$$\frac{\text{Sample ABS}_{\text{FINAL}} - (\text{ABS}_{\text{INITIAL}} * 0.808) * 0.4 * 30}{\text{Standard ABS}_{\text{FINAL}} - (\text{ABS}_{\text{INITIAL}} * 0.808)}$$

NOTE: 0.808 = Correction for Rgt #2 volume  
 0.4= Glycerol Standard (G/L)  
 30 = Sample Dilution Factor

**APPENDIX**

**AUTOMATED TESTING**

ChemWell for Wine uses a new **BLANKING** test procedure, contact Unitech Technical Service for details.

Prepare **Automation Working Reagent** by mixing 4 volumes Reagent #1 with 1 volume Reagent #2 sufficient for the number of samples:

	# of Tests*	20T	36T
Reagent #1		4mL	8mL
Reagent #2		1mL	2mL

\* “# of Tests” accounts for Reagent Bottle dead volume

Working Reagent is stable for 2 weeks when stored refrigerate (or on-board refrigerated 'ChemWell for Wine' system.)

Blank Solution is ready to use and stable until the expiry printed on the label. Load '**Automation Working Reagent**' (above) and **Blanking Solution** in Reagent Rack.

**Calculations:**

'ChemWell for Wine' analyzes blank and reaction cuvettes for each sample and calculates results automatically from either one standard or a multi-point standard curve. ChemWell will dilute and retests values above linear range.

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