

UNITECH SCIENTIFIC CALIFORNIA, U.S.A.

REAGENT™

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.

- Pipet 100 uL standard, samples into cuvettes, as shown on the following table, using micropipettes.
- Add Reagent 1 to each reaction cuvette, mix, and wait, as shown in the Table.
- Zero the spectrophotometer using Reagent Blank Cuvette. Read Initial ABS of Standard and Sample cuvettes.

Pipet into ...	Reagent Blank Cuvette	Std/Sample Cuvettes
DI water	100 µL	
Standard/Sample		100 µL
Reagent #1	2 mL	
Mix and wait 2 minute Zero spectrophotometer with Reagent Blank Read (ABS _{INITIAL}) of each cuvette		
Reagent #2	500 µL	
Mix and wait 10 minutes Read ABS _{FINAL} of each Cuvette		

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

CALCULATION

Calculate the Glycerol concentration:

$$\text{Glycerol G/L} = \frac{\text{Sample ABS}_{\text{FINAL}} - (\text{ABS}_{\text{INITIAL}} * 0.808) * 0.4 * 30}{\text{Standard ABS}_{\text{FINAL}} - (\text{ABS}_{\text{INITIAL}} * 0.808)}$$

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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