PURPOSE OF THE TEST

Glycerol (or glycerin) is a natural by-product of alcoholic fermentation, without aromatic properties but providing a sensation of fullness in the mouth. The content of glycerol is directly related to the degree of maturity of the grape, the microorganisms present and the fermentation process used (temperature, yeast species, nitrogen source).

METHOD

Glycerokinase (GK) catalyzes the phosphorylation of glycerol by the action of adenosine-5'-triphosphate (ATP)

$$Glycerol + ATP \overset{GK}{\rightarrow} Glycerol\text{-}3\text{-}P + \text{ADP}$$

ADP is then reverted into ATP by means of pyruvate kinase (PK) acting over phosphoenolpyruvate releasing pyruvate.

$$ADP + PEP \overset{PK}{\rightarrow} ATP + Pyruvate$$

The pyruvate is then transformed into lactate by Lactate Dehydrogenase consuming $NADH^+$

$$Pyruvate + NADH \overset{LDH}{\longrightarrow} Lactate + NAD$$

The concentration of glycerol is proportional to the decrease of absorbance at 340 nm.

CONTENT

R1	2 x 30 mL	Buffer pH 7.40, ATP 2mM, NADH 8 mM, PEP 45 mM, GK (>200 U/mL), PK (>30 U/mL), LDH (>200 U/mL)
CTRL	1 x 3 mL	Glycerol 0,20 g/L (0,17 – 0,23 g/L)

REAGENT PREPARATION

Reagents are ready to use and are stable up to expiry date as supplied when stored at 2-8 ºC. Do not freeze.

Discard if absorbance of blank is lower than 1.000 OD at 340 nm.

SAMPLES

The samples must be free of turbidity and particles. Centrifuge or filter if necessary. The presence of CO_2 introduces instability in the measure. Samples containing CO_2 must be degassed beforehand. In samples with very high colour intensity, the pigment may interfere with the measurement. Treat with polyvinylpolypyrrolidone (PVPP 0.1g for each 10 mL) to reduce the level of colour.

For manual procedures, dilute sample at 1:30 (1 part of sample and 29 parts of distilled water). Samples with concentration higher than the measurement range at expected predilution can be further diluted with distilled water. Multiply the final result by the final dilution factor.

For automated procedures, state the predilution factor in the corresponding field of the application

PROCEDURE OVERVIEW

Treat standard, controls and samples as Sample. Use distilled water as Blank.

Use WINECAL (code SY2100) or WINECAL RTU (SY2100R) as standard. Standard is ready to use and does not require further dilution for glycerol

For manual use, sample and control should be diluted with distilled water 1:30. Standard is ready to use (no dilution required).

Volumes stated below can be adjusted to other analytical procedures. Expected performance can vary if those ratios S:R1 are not used exactly as stated.

Pipette into a cuvette:

	Blank reaction	Sample/Std Reaction
Reagent 1	900 µL	900 µL
Distilled water	9 µL	--
Sample/Standard	--	9 µL

Mix, incubate at 37ºC for 10 minutes and read absorbance at 340 nm (A_1). Then add into the cuvette:

Concentration of glycerol is calculated as:

$$Glycerol = \frac{(A_1)_{\text{sample}} - (A_1)_{\text{blank}}}{(A_1)_{\text{standard}} - (A_1)_{\text{blank}}} \; x \; 30 \; x \; C \text{ g/L}$$

C is the value of concentration stated in the standard label for glycerol; 30 is the predilution factor

ASSAY PARAMETERS FOR ANALYZER DIONYSOS®

Dionysos model	**150**	**240**
Name	GLYCEROL	
Method	End Point A	
Direction	Increasing	
Main Wavelength	520	
Sec. Wavelength	--	
Sample	3	
Reagent 1	300	
Reagent 2	--	
Calibration	Linear	
Blank cycle [150 | 240]	2 - 2	2 - 2
Reading cycle [150 | 240]	18 - 19	20 - 21
Units	g/L	
Decimals	0.00	
Measure range	0.11 ~ 3.5	
R1 Lim. Abs	10000	
Ratio Dil. Auto.	30	
Vol. Sample Dil. Auto	10	

Procedure is linear up to 12 g/L. (0.4 g/L multiplied by 30 (predilution factor)) Calibrate with a single point using the highest concentration standard or with several points as per your quality procedures.

PERFORMANCE

Limit of quantification (LoQ): 0.3 g/L (=0.11 g/L x Sample predilution)
Limit of linearity: 3.5 g/L (=0.4 g/L x Sample predilution)

NOTES

It is recommended to use wine controls to verify quality of calibration. Each laboratory should establish its own quality criteria for acceptance, as well as proper corrective action procedures in case of rejection.

REFERENCES

1. Compendium of International methods of analysis – OIV, Vol 1&2 (2008). Method OIV-MA-AS312-05. Resolution 377/2009
2. Bermeyer, HU. Methods of Enzymatic Analysis, 2nd Ed. Vol. 1, p. 112-117. Academic Press, Inc. NY. (1974)