PURPOSE OF THE TEST

D-Glucose and D-Fructose are the main reducing sugars present in grapes and other fruits. Its determination in the grape allows to verify their state of maturity to establish the optimum moment of harvest. In the must, allows to estimate the amount of alcohol that will be produced during the fermentation. Finally, at the end of the fermentation, to assess the remaining sugar that could produce an undesired fermentation.

METHOD

Hexokinase (HK) catalyzes the phosphorylation of D-Glucose and D-Fructose by Adenosine-5'-triphosphate (ATP).

$$D\text{-}Glucose + ATP \xrightarrow{HK} Glucose\text{-}6\text{-}phosphate + ADP$$
$$D\text{-}Fructose + ATP \xrightarrow{HK} Fructose\text{-}6\text{-}phosphate + ADP$$

The fructose-6-phosphate is converted to glucose-phosphate by the phosphoglucose isomerase (PGI).

$$Fructose\text{-}6\text{-}phosphate \xrightarrow{PGI} Glucose\text{-}6\text{-}phosphate$$

The glucose-6-phosphate dehydrogenase (G6PDH) catalyzes the oxidation of glucose-6-phosphate to 6-phosphogluconate by reducing $NADP^+$.

$$Glucose\text{-}6\text{-}phosphate + NADP^+ \xrightarrow{G6PDH} Glucose\text{-}6\text{-}phosphate + NADPH + H^+$$

Increase of absorbance at 340 nm associated to NADPH formation is directly proportional to concentration of D-Glucose and D-Fructose of sample.

CONTENT

R1	2 x 30 mL	TEA 100 mM, pH 7.6, ATP 4 mM, $NADP^+$ 3 mM
R2	1 x 15 mL	HK (>0,5 UI/L), G6PDH (>1,8 UI/L), PGI (>8 UI/L)
CTL	1 x 3 mL	Glucose+Fructose control 3,00 g/L (2,55-3,45 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 higher than 0.500 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. Samples with concentration higher than the measurement range must be diluted accordingly with distilled water. Multiply the final result by the dilution factor.

PROCEDURE OVERVIEW

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

Use WINECONTROL (code SD2200 or WINECALRTU (code SY2100R) as standard.

Volumes stated below can be adjusted to other analytical procedures. Expected performance can vary if those ratios S:R1:R2 are not used exactly as stated.

Pipette into a cuvette:

	Blank reaction	Sample/Std Reaction
Reagent 1	720 µL	720 µL
Distilled water	9 µL	--
Sample/Standard	--	9 µL

Mix, incubate at 37ºC for 1 minutes and read absorbance at 340 nm (A_1). Then add into the cuvette:

	Blank reaction	Test Reaction
Reagent 2	180 µL	180 µL

Mix, incubate for 10 minutes at 37ºC and read absorbance at 340 nm (A_2).

Joint concentration of glucose and fructose is calculated as:

$$Glu + Fru = \frac{(A_2 - 0.80xA_1)_{\text{sample}} - (A_2 - 0.80xA_1)_{\text{blank}}}{(A_2 - 0.80xA_1)_{\text{standard}} - (A_2 - 0.80xA_1)_{\text{blank}}} \; x\; C \text{ g/L}$$

Factor 0.80 is used to correct absorbance for dilution after adding reagent 2. C is the value of concentration stated in the standard label for glucose.

ASSAY PARAMETERS FOR ANALYZER DIONYSOS®

Dionysos model	**150**	**240**
Name	GLUCOSE	
Method	End Point A	
Direction	Increasing	
Main Wavelength	340	
Sec. Wavelength	--	
Sample	3	
Reagent 1	240	
Reagent 2	60	
Calibration	Linear	
Blank cycle [150 | 240]	3 - 4	4 - 5
Reading cycle [150 | 240]	21 - 22	31 - 32
Units	g/L	
Decimals	0.00	
Measure range	0,20 ~ 6,00	
R1 Lim. Abs	5000	
Ratio Dil. Auto.	--	
Vol. Sample Dil. Auto	--	

Procedure is linear up to 6 g/L. Calibrate with a single point using the highest concentration standard or with several points as per your quality procedures.

PERFORMANCE

Limit of Quantification (LoQ): 0.20 g/L
Limit of linearity: 6.0 g/L

NOTES

Using a control sample on a regular basis provides information on the calibration status and possible deterioration of the reagent. In case of deviations greater than 15% on the target value, it is advisable to check the calibration status of the test.

REFERENCES

1. Compendium of International methods of analysis – OIV: OIV-MA-AS311-02
2. Bermeyer, HU. Methods of Enzymatic Analysis, 2nd Ed. Vol. 1, p. 112-117. Academic Press, Inc. NY.
3. Resolution OIV-OENO 600-2018. Determination of D-Glucose and D-Fructose in wines by automated enzymatic method. (2018)