SY2405: D-GLUCONIC ACID

ENZYMATIC – GK/6PGDH

FOR USE IN MUST AND WINE SAMPLES

PURPOSE OF THE TEST

D-gluconic acid (together with its cyclic form, D-Glucolactone) allows to evaluate the degree of firmness of the grape. It is produced from glucose by fungi and yeast and its concentration increases proportionally to the degree of over-ripening of the grapes as well as in grapes infected with fungi (for example, of the genus Botrytis). It is highly recommended to measure it when degree of humidity is high along the process of maturation of the grape to adapt the winemaking process accordingly.

METHOD

D-gluconate kinase (GK) catalyzes the reaction of D-gluconic acid with adenosine-5'-triphosphate (ATP) to produce 6-phosphate-D-gluconate, which is transformed to ribulose-5-phosphate by means of D-gluconate-6-phosphate dehydrogenase (6-PGDH) releasing NADPH.

$$D\text{-}Gluconate + ATP \xrightarrow{GK} D\text{-}Gluconate\text{-}6\text{-}P + ADP$$

$$D\text{-}Gluconate\text{-}6\text{-}P + NADP^{+} \xrightarrow{6PGDH} Ribulose\text{-}5\text{-}P + NADPH + H^{+} + CO2$$

Increase of absorbance at 340 nm associated to NADPH formation is directly proportional to concentration of gluconic acid in sample.

D-Gluconolactone can also be measured if sample is treated beforehand in basic medium (pH >11) to revert the cyclic form into D-Gluconate.

CONTENT

R1	2 x 40 mL	TRIS Buffer 100 mM, pH 6.9, $NADP^{+}$ 1.5 mM, ATP 8 mM
R2	1 x 12 mL	GK (>5 U/mL), 6PGDH (>5 U/mL)

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

For use with must and wine 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 0.1 g 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.

To include D-Gluconolactone into measurement treat sample with potassium hydroxide 2 M enough to bring pH > 10 and incubate 10 minutes at 25 ºC. D-Gluconolactone cannot be measured separately from D-Gluconic.

PROCEDURE OVERVIEW

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

Use WINECAL (code SY2100) 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	800 µL	200 µL
Distilled water	16 µL	--
Sample/Standard	--	16 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	80 µL	80 µL

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

Concentration of gluconic acid is calcultated as:

$$D\text{-}Gluconic = \frac{(A_2 - 0.91xA_1)_{\text{sample}} - (A_2 - 0.91xA_1)_{\text{blank}}}{(A_2 - 0.91xA_1)_{\text{standard}} - (A_2 - 0.91xA_1)_{\text{blank}}}\ x\ C\ \text{g/L}$$

Factor 0.91 is used to correct absorbance for dilution after adding reagent 2. C is the value of concentration stated in the standard label for gluconic.

ASSAY PARAMETERS FOR ANALYZER Y15/Y25®

GENERAL	Name	GLUCONIC
	Analysis mode	Diff. Bi-reagent
	Sample type	ST1
	Units	g/L
	Reaction type	Increasing
	Decimals	2
	Replicates	1
PROC.	Reading	Monochromatic
	Sample	4
	Reagent 1	200
	Reagent 2	20
	Wash	1,2
	Predilution factor	--
	Postdilution factor	2
	Main wavelenght	340
	Ref. wavelenght	--
	Reading 1 at	24 s
	Reading 2 at	600 s
	Add Reagent 2 at	48 s
CAL.	Calibration type	Multiple
	Standard replicates	3
	Blank replicates	3
	Calibration curve	Linear regression
OP.	Limit abs. Blank	0.500
	Limit Blank kinetic	--
	Limit linearity	2.10

Procedure is linear up to 2.10 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): 0.06 g/L

Limit of linearity: 2.10 g/L

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).
2. Bermeyer, HU. Methods of Enzymatic Analysis, 2nd Ed. Vol. 1, p. 112-117. Academic Press, Inc. NY.