# ENZYMATIC TEST KIT FOR THE DETERMINATION OF D-GLUCONIC ACID IN GRAPE JUICE AND WINE

PRODUCT
Product no. 4A130, **for 30 tests, for *in vitro* use only.**

PRINCIPLE OF MEASUREMENT
Free D-Gluconic acid (D-gluconate) is an important metabolite of *Botrytis cinerea*, and is the best marker to estimate the level of infection. D-glucono-δ-lactone is a cyclic ester of D-gluconic acid. It exists in equilibrium with the D-gluconic acid and can represent between 2-10% of the acid level in musts from botrytized grapes (1). D-Gluconic acid (D-gluconate) is phosphorylated to D-gluconate-6-phosphate by adenosine-5'-triphosphate (ATP) in the presence of the enzyme gluconate kinase with the simultaneous formation of adenosine-5'-diphosphate (ADP):

$$\text{D-Gluconate + ATP} \quad \overset{\text{GNTK}}{\rightarrow} \quad \text{gluconate-6-P + ADP}$$
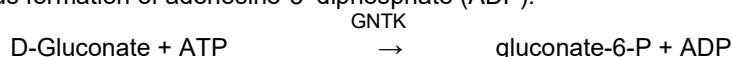
In the reaction catalyzed by 6-phosphogluconate dehydrogenase (6-PGDH), D-gluconate-6-phosphate is oxidatively decarboxylated by nicotinamideadenine dinucleotide phosphate (NADP) to ribulose-5-phosphate with the formation of reduced nicotinamide-adenine dinucleotide phosphate (NADPH):

$$\text{Gluconate-6-P + NADP}^+ \quad \overset{\text{6-PGDH}}{\rightarrow} \quad \text{ribulose-5-P + NADPH + CO}_2 + \text{H}^+$$

The amount of NADPH formed in the above reaction is stoichiometrically related to the amount of D-gluconate. The increase in NADPH is measured at 340nm (2).

CONTENTS
The kit includes the following reagents:

Reagent No.	Reagent	Preparation	Quantity	Stability
1	Buffer	To activate the Buffer, add the contents of Reagent No.2 Coenzymes (ATP/NADP) and mix with inversion until completely dissolved.	33 mL	All reagents (as provided) are stable for 18 months at 4°C or until the kit's expiry date, whichever occurs first. Reagent 1 (Buffer) is stable for 6 months at 4°C once activated or until the kit's expiry date, whichever occurs first.
2	Coenzymes (ATP/NADP)		0.2 g	
3	6-PGDH	Swirl gently before use	0.7 mL	
4	GNTK	Swirl gently before use	0.7 mL	
5	Standard	Nil	3.3 mL	

The shelf life of Reagent 1 & 2 can be extended by placing aliquots in a freezer.
Do not freeze enzyme reagents 3 & 4.
Failure to store reagents at the recommended temperature will reduce their shelf life.
For concentration of Standard, refer to label on bottle.

SAFETY

- Wear safety glasses

- Do not ingest Buffer or Standard as they contain sodium azide as a stabilizer

PROCEDURE
Operating Parameters
Wavelength   340 nm
Cuvettes   1cm, quartz, silica, methacrylate or polystyrene
Temperature   20 – 25˚C
Final volume in cuvette   3.04 mL
Zero   against air without cuvette in light path

SAMPLE PREPARATION

Samples should be diluted with distilled water to ensure that the concentration in the assay solution is no more than 0.6 g/L. For the majority of wine samples, a 1 in 10 dilution is satisfactory.

As a general guide, further dilution is required if the absorbance reading is greater than 1 absorbance unit. Samples may be used directly without decolourisation. Turbid samples should be filtered through Whatman No. 1 filter paper.

To determine the total D-gluconic acid present in juice and wines, D-glucono-δ-lactone must first be hydrolysed by adjusting the sample pH to 10-11 with 2M KOH and incubating for 5-10mins at room temperature. Adjust the pH to 7.5-8.0 with 1M HCl before assaying. The D-glucono-δ-lactone is converted to free D-gluconic acid and is determined together with the original free D-gluconic acid (total D-gluconic acid).

SAMPLE ANALYSIS

a. Pipette the following volumes of reagents into the cuvettes:

Reagent	Blank assay	Standard assay	Samples
1. Buffer/Coenzymes	1.00 mL (1000 µL)	1.00 mL (1000 µL)	1.00 mL (1000 µL)
Distilled water	2.00 mL (2000 µL)	1.90 mL (1900 µL)	1.90 mL (1900 µL)
3. 6-PGDH	0.02 mL (20µL)	0.02 mL (20µL)	0.02 mL (20µL)
Sample or Standard		0.10 mL (100 µL)	0.10 mL (100 µL)

b. Mix well and read absorbances, A_1, after approximately 5 minutes.

c. Pipette the following reagent into the cuvettes:

4. GNTK	0.02 mL (20µL)	0.02 mL (20µL)	0.02 mL (20µL)

d. Mix well and read absorbances, A_2, once reaction is complete (approximately 20 minutes).

CALCULATIONS*

1. Calculate the Net Absorbance for the Blank, Sample and Standard:

$$\text{Net Absorbance, } A_N \quad = \quad A_2 - A_1$$

2. Calculate the Corrected Absorbance by subtracting the Net Absorbance for the Blank from the Net Absorbance for the Sample.

$$\text{Sample Corrected Absorbance, } A_C \quad = \quad \text{Sample } A_N - \text{Blank } A_N$$

3. Do the same for the Standard by substituting the Standard absorbances in place of the Sample absorbances.

4. Calculate the D-Gluconic acid concentration as follows;

$$\text{D-Gluconic Acid Concentration (g/L)} \quad = \quad A_C \times 0.9465 \times \text{Dilution Factor}$$

*A calculation spreadsheet is available for download at the following locations

Australia based users
https://winechek.com/calculation-worksheets/

Users outside of Australia
http://www.vintessential.com.au/resources/calculation-worksheets/

REFERENCES

1. Barbe, J.C. *et al* 2002, Journal of Agricultural and Food Chemistry 11/2002; 50 (22) :pp. 6408-6412
2. Bergmeyer, H.U. *et al* 1984, *Methods of Enzymatic Analysis*, 3rd ed., vol. 6, pp. 220-227; Verlag Chemie, Weinheim.