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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 represents 4.1% of the acid level (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):

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

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):

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

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	33 mL	18 months at 4°C (6 months once activated)
2	Coenzymes (ATP/NADP)	Coenzymes (ATP/NADP) and mix with inversion until completely dissolved.	0.2 g	18 months at 4°C
3	6-PGDH	Swirl gently before use	0.7 mL	18 months at 4°C
4	GNTK	Swirl gently before use	0.7 mL	18 months at 4°C
5	Standard	Nil	3.3 mL	18 months at 4°C

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:

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

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

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

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;

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

*A calculation spreadsheet is available for download at:
http://www.vintessential.com.au/certification/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.