

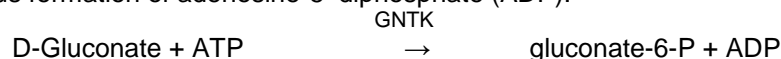
## ENZYMATIC TEST KIT FOR THE DETERMINATION OF D-GLUCONIC ACID IN GRAPE JUICE AND WINE

### PRODUCT

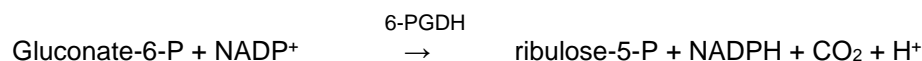
Product no. 4A130, for 30 tests, for *in vitro* use only.

### 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- $\delta$ -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):



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):



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- $\delta$ -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- $\delta$ -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 $\mu$ L)	1.00 mL (1000 $\mu$ L)	1.00 mL (1000 $\mu$ L)
Distilled water	2.00 mL (2000 $\mu$ L)	1.90 mL (1900 $\mu$ L)	1.90 mL (1900 $\mu$ L)
3. 6-PGDH	0.02 mL (20 $\mu$ L)	0.02 mL (20 $\mu$ L)	0.02 mL (20 $\mu$ L)
Sample or Standard		0.10 mL (100 $\mu$ L)	0.10 mL (100 $\mu$ L)

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 (20 $\mu$ L)	0.02 mL (20 $\mu$ L)	0.02 mL (20 $\mu$ L)
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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*, 3<sup>rd</sup> ed., vol. 6, pp. 220-227; Verlag Chemie, Weinheim.