

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

**PRODUCT**

Product no. 4A130, for 60 tests, for *in vitro* use only.

**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	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)	Coenzymes (ATP/NADP) and mix with inversion until completely dissolved.	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 <i>micro-cuvette</i> , quartz, silica, methacrylate or polystyrene Re-ordering code 2C890
Temperature	20 – 25°C
Final volume in cuvette	1.52 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	500 µL	500 µL	500 µL
Distilled water	1000 µL	950 µL	950 µL
3. 6-PGDH	10 µL	10 µL	10 µL
Sample or Standard		50 µL	50 µL

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

c. Pipette the following reagent into the cuvettes:

4. GNTK	10µL	10µL	10µL
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d. Mix well and read absorbances,  $A_2$ , once reaction is complete (approximately 20 minutes).

### CALCULATIONS\*

These may be performed on the Absorbance one software directly, or using the calculation spreadsheets below\*

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 the following locations in the absence of Absorbance one software.

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*, 3<sup>rd</sup> ed., vol. 6, pp. 220-227; Verlag Chemie, Weinheim.