ENZYMATIC TEST KIT FOR THE DETERMINATION OF AMMONIA IN GRAPE JUICE AND WINE

PRODUCT
Product no. 4A120, **for 30 tests, for *in vitro* use only**

PRINCIPLE OF MEASUREMENT
Ammonia is found in both grape juice and wine. It is an important nutrient for yeast during primary fermentation. It is determined enzymatically according to the following equation:

$$\alpha\text{-ketoglutarate} + NADH + NH_4^+ \xleftrightarrow{GlDH} L\text{-glutamate} + NAD^+ + H_2O$$

Ammonia reacts with α-ketoglutarate and reduced nicotinamide adenosine dinucleotide (NADH) in the presence of glutamate dehydrogenase (GlDH) to form L-glutamate and NAD. The amount of NADH consumed is measured at 340 nm and is stoichiometrically related to the amount of ammonia present.
The **Yeast Available Nitrogen** (YAN) content of the juice can be determined by adding the Ammonia Nitrogen (AN) content to the Primary Amino Acid Nitrogen (PAAN) content.
PAAN can be determined by Vintessential Laboratories Analysis Kit 4A110.

CONTENTS
The kit includes the following reagents:

Reagent No.	Reagent	Preparation	Quantity	Stability
1	Buffer	Nil	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 2 (NADH) is stable for 1 month at 4°C once dissolved or until the kit's expiry date, whichever occurs first.
2	NADH	Add 1.7 mL of distilled water to either bottle as required, mix to dissolve	2 x 1.7 mL	
3	GlDH	Mix gently by inversion before use	0.7 mL	
4	Standard	Nil	3.3 mL	

The shelf life of Reagent 1 can be extended by placing aliquots in a freezer.
Do not freeze reagents 2 or 3.
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	3.12 mL
Zero	against air without cuvette in light path

SAMPLE PREPARATION

Samples should be diluted with distilled water to ensure concentration in the assay solution is no more than 40 mg/L (ppm). Ideally, A_1 should lie between 0.90 – 1.20 absorbance units.
For samples with less than 200 mg/L of Ammonia, a 1 in 5 dilution should be sufficient.
For samples containing between 200 – 400 mg/L of Ammonia, a 1 in 10 dilution would be appropriate.

Undiluted red wines or highly coloured undiluted juice samples require decolourisation.
To decolourise, add approximately 0.1 g of PVPP to 5 mL of sample in a test tube. Shake well for about 1 minute. Clarification is achieved by settling or filtering through Whatman No. 1 filter paper.

SAMPLE ANALYSIS

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

Reagent	Blank	Standard	Sample
1. Buffer	1.00 mL (1000 µL)	1.00 mL (1000 µL)	1.00 mL (1000 µL)
2. NADH	0.10 mL (100 µL)	0.10 mL (100 µL)	0.10 mL (100 µL)
Distilled water	2.00 mL (2000 µL)	1.90 mL (1900 µL)	1.90 mL (1900 µL)
Sample/Standard		0.10 mL (100 µL)	0.10 mL (100 µL)

b. Mix well and read absorbances, A_1, once constant (approximately 5 minutes).

c. Pipette the following reagent into the cuvettes:

3. GlDH	0.02 mL (20µL)	0.02 mL (20µL)	0.02 mL (20µL)

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

CALCULATIONS*

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

Net Absorbance, A_N = $A_1 - A_2$

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

Sample Corrected Absorbance, A_C = Sample A_N – Blank A_N

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

4. Calculate the Ammonia concentration as follows;

Ammonia (mg/L) = A_C x 84.3 x Dilution Factor

5. Calculate Ammonia Nitrogen as follows;

Ammonia Nitrogen (mg/L) = Ammonia (mg/L) x 0.82

To calculate YAN (Yeast Assimilable Nitrogen), simply add Ammonia Nitrogen (AN) to the Primary Amino Acid Nitrogen (PAAN) calculated from kit 4A110:

YAN = PAAN + AN

*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. Bergmeyer, H.U. *et al* 1985, *Methods of Enzymatic Analysis*, 3rd ed., vol. 8, pp. 454-461; Verlag Chemie, Weinheim.