

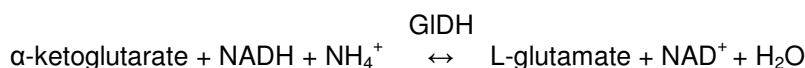
## ENZYMATIC ANALYSIS KIT FOR THE DETERMINATION OF AMMONIA IN GRAPE JUICE AND WINE

### 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:



Ammonia reacts with  $\alpha$ -ketoglutarate and reduced nicotinamide adenosine dinucleotide (NADH) in the presence of glutamate dehydrogenase (GIDH) 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	18 months at 4°C
2	NADH	Add 3.3 mL of distilled water, mix to dissolve	3.3 mL	18 months at 4°C (1 month at 4°C once diluted)
3	GIDH	Mix gently by inversion before use	0.7 mL	18 months at 4°C
4	Standard	Nil	3.3 mL	18 months at 4°C

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 80 mg/L (ppm). Ideally,  $A_1$  should lie between 1.0 – 1.5AU. For samples with less than 400 mg/L, a 1 in 5 dilution should be sufficient. From our experience, most juice samples will not require dilution.

As a general guide, further dilution is required if the sample *net* absorbance,  $A_N$ , is greater than 1 absorbance unit.

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 $\mu$ L)	1.00 mL (1000 $\mu$ L)	1.00 mL (1000 $\mu$ L)
2. NADH	0.10 mL (100 $\mu$ L)	0.10 mL (100 $\mu$ L)	0.10 mL (100 $\mu$ L)
Distilled water	2.00 mL (2000 $\mu$ L)	1.90 mL (1900 $\mu$ L)	1.90 mL (1900 $\mu$ L)
Sample/Standard		0.10 mL (100 $\mu$ L)	0.10 mL (100 $\mu$ L)

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

c. Pipette the following reagent into the cuvettes:

3. GIDH	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 (approx 20 minutes).

## CALCULATIONS\*

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

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

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

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

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

4. Calculate the Ammonia concentration as follows;

$$\text{Ammonia (mg/L)} = A_C \times 84.3 \times \text{Dilution Factor}$$

5. Calculate Ammonia Nitrogen as follows;

$$\text{Ammonia Nitrogen (mg/L)} = \text{Ammonia (mg/L)} \times 0.82$$

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

$$\text{YAN} = \text{PAAN} + \text{AN}$$

\*A calculation spreadsheet is available for download at [www.vintessential.com/enzyme\\_kits.htm](http://www.vintessential.com/enzyme_kits.htm)

## REFERENCES

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