

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

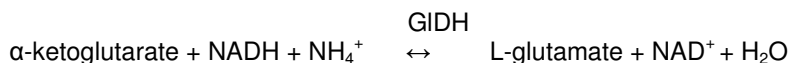
FOR DISCRETE ANALYSERS

PRODUCT

Product no. 4B120, 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 α -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 340nm and is stoichiometrically related to the amount of ammonia present. The **Yeast Assimilable 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 Discrete Analyser Kit 4B110.

CONTENTS

The kit includes the following reagents:

Reagent No.	Reagent	Quantity	Stability
REAGENT 1	Buffer	19.5 mL x 2	24 months at 4°C
REAGENT 2	NADH	19.5 mL x 2	6 months at 4°C
REAGENT 3	GIDH	13.2 mL x 2	12 months at 4°C

The shelf life of Reagent 1 can be extended by placing aliquots in a freezer. Do not freeze reagents 2 & 3. Failure to store reagents at the recommended temperature will reduce their shelf life.

SAFETY

- **Wear safety glasses, Reagent R1 is mildly corrosive**
- **The reagents contain sodium azide as preservative. Do not ingest.**

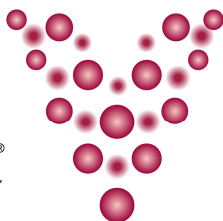
PROCEDURE

Reagent Definition

Reagent	Amm R1	Amm R2	Amm R3
Stable on board (days)	1	1	1
Alarm limit (mL)	1.0mL	1.0mL	1.0mL
Vial volume	20mL	20mL	20mL
Syringe speed	Normal	Normal	Normal

Test Definition

Test type	Photometric
Full name	Ammonia
Result unit	mg/l
Number of decimals	2
Acceptance	Manual
Dilution 1+	0
Sample type	Wine, Must, Juice



Calibration Parameters

Calibration type	Linear
Repeat time (d)	1
Points/Calibrator	Duplicate
Acceptance	Manual
Curve direction	Descending
Type of calibrators	Separate

Calibrator	Conc. (mg/l)	Dil. Ratio 1+
Water	0	0
Amm 35	35	0
Amm 70	70	0
Amm 100	100	0

Test Flow

Reagent	Reagent	Sample	Incubation	Blank	Reagent	Incubation	End point
Reagent	Reagent	Volume (µl)	Time (sec.)	Resp. min	Reagent	Time (sec.)	Wavelength(nm)
Amm R1	Amm R2	3	600	*	Amm R3	600	340
Volume (µl)	Volume (µl)	Disp. with		Resp. max	Volume (µl)		Side wavel.(nm)
65	65	Extra		*	40		NONE
Disp. with	Disp. with	Volume (µl)			Disp. with		
Extra	Extra	10			Extra		
Volume (µl)	Volume (µl)	Wash reagent			Volume (µl)		
10	10	NONE			10		
Wash reagent	Wash reagent				Wash reagent		Meas. type
Water	Water				Water		Fixed timing

CALCULATIONS

To determine Ammonia Nitrogen (AN) content, multiply the Ammonia content by a factor of 0.82 as per below:

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

To calculate Yeast Assimilable Nitrogen (YAN), add the Ammonia Nitrogen (AN) to the Primary Amino Acid Nitrogen (PAAN) determined from kit 4B110:

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

REFERENCES

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