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ANALYSIS KIT FOR THE DETERMINATION OF PRIMARY AMINO ACID NITROGEN IN GRAPE JUICE

PRODUCT

Product no. 4A110, **for 30 tests**.

PRINCIPLE OF MEASUREMENT

During fermentation of grape juice yeasts require a source of nitrogen as a nutrient. Primary amino acids provide a portion of this nitrogen requirement. This test kit is suitable for measuring the Primary Amino Acid Nitrogen (PAAN) content in grape juice and non-fermenting must. In this kit Ortho-phthaldialdehyde (OPA) and N-acetyl-L-cysteine (NAC), in the presence of an alkaline buffer, bind with primary amino acids to form coloured complexes whose absorbance is measured at 335 nm by a UV/VIS spectrophotometer[1].

The **Yeast Available Nitrogen** (YAN) content of the juice can be determined by adding this PAAN content to the Ammonia Nitrogen (AN) content.

AN can be determined by Vintessential Laboratories Enzymatic Analysis Kit 4A120.

CONTENTS

The kit includes the following reagents:

Reagent No.	Reagent	Preparation	Quantity	Stability
1	Buffer	Nil	2 x 33 mL	Stable
2	NAC	Add 30 mL of distilled water, mix to dissolve	30 mL	2 years at 4°C (2 months at 4°C diluted)
3	OPA	Add contents of Bottle No.4, Ethanol, mix to dissolve	3.3 mL	2 years at 4°C (2 months at 4°C diluted)
4	Ethanol	Nil	3.3 mL	Stable
5	Standard	Nil	3.3 mL	2 years at 4°C

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**
- **Reagent 1 is alkaline**
- **Reagent 4 is a flammable solvent**
- **Do not ingest Standard as it contains sodium azide as a stabilizer**

PROCEDURE

Operating Parameters

Wavelength	335nm
Cuvettes	1cm, quartz, silica, methacrylate or polystyrene
Temperature	20 – 25°C
Final volume in cuvette	3.05 mL
Zero	against air with no cuvette in light path

SAMPLE PREPARATION

Samples should be refrigerated upon receipt or frozen until testing. Dilute juice with distilled water 1:1 if PAAN is likely to be high. Must samples may need dilution if fermentation is just beginning.

Filter very cloudy samples; highly coloured samples may 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	2.00 mL (2000 µL)	2.00 mL (2000 µL)	2.00 mL (2000 µL)
2. NAC	0.90 mL (900 µL)	0.90 mL (900 µL)	0.90 mL (900 µL)
Distilled water	0.05 mL (50 µL)		
Sample/Standard		0.05 mL (50 µL)	0.05 mL (50 µL)

b. Mix well and read absorbances, A_1.

c. Pipette the following reagent into the cuvettes:

3. OPA	0.10 mL (100 µL)	0.10 mL (100 µL)	0.10 mL (100 µL)

d. Mix well and read absorbances, A_2, after 10 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 amount of Primary Amino Acid Nitrogen of the Samples using the formula below:

$$\text{Primary Amino Acid Nitrogen (mg N/L)} = A_C \times 130 \times \text{dilution factor}$$

To calculate YAN (Yeast Assimilable Nitrogen), simply add Primary Amino Acid Nitrogen (PAAN) to the Ammonia Nitrogen (AN) calculated from kit 4A120:

$$YAN = PAAN + AN$$

*A calculation spreadsheet is available for download at www.vintessential.com/enzyme_kits.htm

REFERENCES

1. Dukes, B.C. and Butzke, C.E. 1998, “Rapid determination of primary amino acids in grape juice using an o-phthaldialdehyde/N-acetyl-L-cysteine spectrophotometric assay”, *Am.J.Enol.Vitic*, Vol 49, No.2, pp. 125-134.