ABSORBANCE ONE TEST KIT FOR THE DETERMINATION OF PRIMARY AMINO ACID NITROGEN IN GRAPE JUICE

PRODUCT
Product no. 4A110, **for 60 tests**.

The **Yeast Assimilable 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 Enzymatic Analysis Kit 4A120.

CONTENTS
The kit includes the following reagents:

Reagent No.	Reagent	Preparation	Quantity	Stability
1	Buffer	Ready to use	2 x 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 (NAC) is stable for 6 months at 4°C once dissolved or until the kit's expiry date, whichever occurs first.
2	NAC	Add 30 mL of distilled water & mix until dissolved	30 mL	
3	OPA	Ready to use	3.3 mL	
4	Standard	Ready to use	3.3 mL	

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

Do not ingest Standard as it contains sodium azide as a stabilizer

PROCEDURE
Operating Parameters

Wavelength	340nm
Cuvettes	1cm *micro-cuvette*, quartz, silica, methacrylate or polystyrene Re-ordering code 2C890
Temperature	20 – 25°C
Final volume in cuvette	1.53 mL
Zero	against air with no cuvette in light path

SAMPLE PREPARATION
Samples should be refrigerated upon receipt or frozen until testing. Dilute juice or must samples with distilled water 1:1 if PAAN is likely to be in the range of 130-260mg/L. Dilution with distilled water 1:4 (x5 dilution factor) will allow the detection of PAAN up to 650mg/L.

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.
Avoid the use of activated charcoal.

SAMPLE ANALYSIS

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

Reagent	Blank assay	Standard assay	Sample assays
1. Buffer	1000 µL	1000 µL	1000 µL
2. NAC	450 µL	450 µL	450 µL
Distilled water	25 µL		
Sample or Standard		25 µL	25 µL

b. Mix well and read absorbances, A_1.

c. Pipette the following reagent into the cuvettes:

3. OPA	50 µL	50 µL	50 µL

d. Mix well, incubate for 10 minutes and read absorbances, A_2

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:

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:

Sample Corrected Absorbance, A_C = Sample A_N – 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 in the Samples using the formula below:

Primary Amino Acid Nitrogen (mg N/L) = AC x 130 x 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 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. 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.