

1^o AMINO NITROGEN

OPA/NAC UV- fast Sample Blanking Method

Product #:

PAN-60 (30-Tests)
PAN-150 (75-Tests)
PAN-500 (250 Tests)

INTENDED USE

Primary (1^o) Amino Nitrogen UniTAB™ Reagent is intended for measuring nitrogen content of primary [or *alpha*] amino groups in wine. Primary Amino Nitrogen plus Ammonia Nitrogen content equals Yeast Assimilable Nitrogen Content of wine.

KIT CONTENTS

	30T	75T	250T
NAC Reagent Tablets	12	30	100
OPA Reagent	6.5mL	16mL	2x27mL
Standard 120 mg/L	1mL	1mL	5mL

SYSTEM REQUIREMENT

Spectrophotometer should be capable of reading 340 nm absorbance over a 0-2 A range with a 1 cm lightpath.

SAMPLES

If wine samples are visually clear, no sample pretreatment is needed. Filter or centrifuge turbid samples, e.g. juice, must or fermentation samples.

REAGENTS

OPA Reagent and Standards are ready to use (no preparation needed.) Kit contents are stable through the labeled expiration date when stored at 2-8 °C; store tablets tightly sealed with the desiccant pack provided.

ASSAY PREPARATION

Working Reagent

Prepare Working Reagent just prior to testing. Calculate the volume required (2mL X # of tests [i.e. blank + standard + wine samples]). For Automated W-Rgt requirement, refer to Automated Testing Section below.

Dissolve each Reagent Tablet in 5mL deionized (DI) water using clean glassware. Mix by gentle inversion. Working reagent is stable for 1 day refrigerated; allow to reach room temperature prior to assay.

TESTING PROCEDURE

- Pipet water into the Reagent Blank cuvette; pipet standards and samples into respective cuvettes.
- Pipet Working Reagent into cuvettes.
- Zero spectrophotometer with Reagent Blank. Read initial absorbance (A₀) values for Std. and Samples.
- Wait 3 minutes and read initial absorbance (A₁).
- Add OPA Reagent, mix and wait 10-20 minutes; read final absorbance (A₂).

Pipet into Cuvettes	Reagent Blank Cuvette	Sample or Standard Cuvettes
Sample		20 µL
DI water	20 µL	
Working Reagent	2 mL	2 mL
Mix and wait 3 minutes. Zero spectrophotometer with Reagent Blank Read A _{INITIAL} (Initial absorbance)		
OPA Reagent	200 µL	200 µL
Mix and wait 10 min. Read A _{FINAL} (Final absorbance).		

The testing range is up to 400 mg/L. If a calculated result is over-range, dilute sample and retest, multiply this test result by the dilution factor.

CALCULATIONS

1. Our online "Flex Calculator™ PAN" spreadsheet at <http://unitechscientific.com/calculators.htm> is available for download.

PAN mg/L values will be calculated automatically.

2. Manual Calculation:

Calculate ΔA values and mg/L as follows for each cuvette:

$$\Delta A = A_{\text{FINAL}} - A_{\text{INITIAL}}$$

Subtract the ΔA of the Reagent Blank from each sample and standard the ΔA:

$$\text{Net A} = \Delta A_{\text{SAMPLE}} - \Delta A_{\text{BLANK}}$$

Calculate Concentration (based on 120 mg/L Standard)

$$\text{PAN mg/L} = (120) \times (\text{d.f.}) \times \frac{\text{Net A}_{\text{SAMPLE}}}{\text{Net A}_{\text{STANDARD}}}$$

Refer to SIGNIFICANCE OF MEASUREMENTS for definition of YANC (Yeast Assimilable Nitrogen Compounds.)

QUALITY CONTROL

Test the standard in each assay to calculate wine results (as above, by Standard Method.)

If using the Alternate Calculations (Appendix, below) test the standard to monitor assay performance. Performance is acceptable if result of standard is within 15% of labeled value. Factors that may affect the performance of this test include instrument function, temperature, glassware cleanliness, and pipetting accuracy (use calibrated micropipettors.)

APPENDIX

NOTES FOR ALTERNATE CALCULATIONS:

Multi-point standard curve Sample concentrations are calculated from the best-fit standard curve. Standard sets available from Unitech Scientific LLC.

METHODOLOGY & CHEMICAL PRINCIPLES

The assay method for primary amino nitrogen is based on that reported by Dukes and Butzke and others. Primary amino groups are derivatized by o-phthaldialdehyde and N-acetyl-L-cysteine (OPA/NAC) to form isoindoles which are detected spectrophotometrically at 340 nm. Neither amino nitrogen not assimilable by yeast (e.g. acylated or blocked amines, proline and hydroxyproline) nor ammonia nitrogen are not detected in this reaction; hence YANC requires independent assays of primary amino nitrogen and ammonia nitrogen.

SIGNIFICANCE OF MEASUREMENTS

Primary amino groups and ammonia* are nitrogen sources for yeast. Together they comprise Yeast assimilable Nitrogen compounds (YANC) which, in optimal concentrations (e.g. 200-350 mg Nitrogen/L) promote rapid, clean fermentation during table wine production.

If the concentration of YANC is too low, the fermentation may be incomplete or sulfides may be generated. In the presence of ethanol, too high a YANC, specifically a elevated L-arginine levels, may result in the formation of ethyl carbamate, a potential carcinogen.

*Determine Ammonia nitrogen using Unitech Ammonia Reagent.

AUTOMATED TESTING

'ChemWell for Wine' analysis is linear to 440 mG/L. Use the tables below to determine the required volumes of Reagents:

	<u>25T</u>	<u>70T</u>
NAC Tablets	2	4
Deionized Water	<u>10mL</u>	<u>20mL</u>
(# of Tests accounts for Reagent Bottle dead volume)		
OPA Reagent (aliquot)	1.4mL	2.8mL

Placed the Working Reagent and OPA Reagent in CW reagent rack.

CALCULATIONS:

'ChemWell for Wine' calculates results automatically from either one standard or a multi-point standard curve; dilutes and retests values above linear range.

TRADEMARKS:

"ChemWell for Wine", "Flex Calculator", "UniTAB" are Trademarks of Unitech Scientific LLC

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