

UNITAB™ REAGENT

1° AMINO NITROGEN

OPA/NAC UV- fast Sample Blanking Method

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

INTENDED USE

Unitech Scientific 1°Amino Nitrogen Reagent is intended for the determination of nitrogen from the primary [or *alpha*] amino groups in wine and other liquid samples.

METHODOLOGY & CHEMICAL PRINCIPLES

The assay method for primary amino nitrogen is based on that reported by Dukes and Butzke¹ and others.^{2,3} Primary amino groups are derivatized by o-phthaldialdehyde and N-acetyl-L-cysteine (OPA/NAC) to form isoindoles. These derivatives are detected spectrophotometrically at 340 nm. This "Blanking Method" permits sample blanking of each reaction cuvette.

Yeast non-assimilable amino nitrogen (e.g. acylated or blocked amines, proline and hydroxyproline) and ammonia nitrogen are not detected in this reaction.¹ YANC determination therefore requires independent assays of primary amino nitrogen and ammonia nitrogen.

REAGENTS

Active Ingredients are:	Concentration as Formulated	Quantity/Kit		
		Pan-60	-150	-500
1. <u>NAC Reagent Tablets</u>		12	30	100
N-Acetyl-L-cysteine	5mM			
[buffered pH 9.5]				
2. <u>OPA Reagent</u>				
o-Phthaldialdehyde	50mM	6.5	16	54 mL
3. <u>Standard</u>	<u>mg Nitrogen/L</u>	<u>mM L-Arginine</u>		
	120	8.56	1	1 5 mL

Available from Unitech: **L-Arginine High Standards**

Primary Amino Nitrogen: 20, 60, 120, 200, 400 mg-N/L (equivalent to 250, 750, 1500, 2500, 5000 mg/L L-Arg.) @5mL/Level.

STORAGE & REAGENT PREPARATION

Kit components are stable until the labeled expiration date when stored in original container at 2-8°C; store tablets tightly sealed with desiccant pack provided. Prepare sufficient WRgt for all samples and standards in the assay. Use clean glassware, mix by gentle inversion for about 10 minutes. Examples are provided in the table below.

MANUAL TESTING	<u>5 Tests</u>	<u>10 Tests</u>	<u>20 Tests</u>
NAC Tablets	2	4	8
Deionized Water	10mL	20mL	40mL

CHEMWELL (AUTOMATED)	<u>30 Tests</u>	<u>80 Tests</u>	<u>180 Tests</u>
NAC Tablets	2	4	8
Deionized Water	10mL	20mL	40mL
(# of Tests accounts for Reagent Bottle dead volume)			

NAC Solution may be used for 5 days or more when stored refrigerated in sealed glass containers.

PROCEDURE

System requirements: Wavelength 335 - 340 nm, absorbance range 0-2.5 pathlength 1.0 cm. For alternative sample volume, etc., refer to NOTES.

Pipet into ...	Zero Cuvette	Sample Cuvette	Standard Cuvettes
DI Water	20µL		
Sample		20 µL	
Standard			20 µL
NAC Solution	2 mL		
Mix and wait 3 minutes. Zero spectrophotometer with DI water. Read initial absorbance (A _I) of each cuvette.			
OPA Solution	200 µL		
Mix and wait 15minutes. Read final absorbance (A _F) of each cuvette.			

1. Label one cuvette for Zero, Standard, and each Sample.
2. Pipet water into the "Zero" cuvette, and standard & samples into appropriate cuvettes as shown in the table above.
3. Pipet 2.0mL of NAC Solution into each cuvette. Mix and wait 3 minutes.
4. Zero spectrophotometer and read initial absorbance of each cuvette.
5. Pipet 200µL OPA Solution into each cuvette. Mix and wait 15minutes. Read final absorbance of each cuvette.

If the absorbance value of any reaction mixture exceeds the instrument absorbance limit, dilute mixture 1 to 1 with distilled water; multiply the resulting absorbance by "2" (the dilution factor). This reagent is linear to 400 mg/L.

CALCULATION

1. Calculate Net Absorbance for each cuvette:
Net A = (A_F - A_I) - Zero (A_F - A_I)
2. Calculate results for each sample as follows:

$$\begin{aligned}
 &1^\circ\text{Amino Nitrogen, (mg Nitrogen/L)} \\
 &= \frac{\text{Conc. Std}}{\text{Net A}_{\text{STD}}} \times \text{Net A}_{\text{SAMPLE}} \\
 &= \frac{120}{\text{Net A}_{\text{STD}}} \times \text{Net A}_{\text{SAMPLE}}
 \end{aligned}$$

YANC = Ammonia* Nitrogen + 1°Amino Nitrogen

* Refer to Directions for Unitech's Ammonia kit

SIGNIFICANCE OF MEASUREMENT

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^{4,5} 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 carbamate⁶, a potential carcinogen.

REFERENCES

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4. Bely, M., J.-M. Sablayrolles, and P. Barre (1990) Automatic detection of assimilable nitrogen deficiencies during alcohol fermentation in enological conditions. *J. Ferm. Bioeng.* **70**, 246-252.
5. Henschke, P.A. (1996) Hydrogen sulfide production by yeast during fermentation. In: *Association for Winery Technology and International Management. 11th International Oenological Symposium*. Sopron, Hungary. June 3-5, pp. 84-102.
6. Daudt C., C. Ough, D. Stevens, T. Herraiz (1992) Investigations into ethyl carbamate, N-propyl carbamate, and urea in fortified wines. *Am. J. Enol. and Vit.* **43**(4), pp. 318-322.

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