UNITECH SCIENTIFIC CALIFORNIA, U.S.A.

UNITAB™ REAGENT

AMMONIA

Enzymatic, UV-Method

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

INTENDED USE

Ammonia UniTAB™ Reagent is intended for measuring ammonia concentrations in wine. The calculated Ammonia Nitrogen plus Primary Amino Nitrogen equals Yeast Assimilable Nitrogen (YAN) Content of wine.

KIT CONTENTS

	30T	75T	250T
Ammonia Reagent Tablets	12	30	100
AMM (GLDH) Trigger Enzyme	1.3 mL	3.3 mL	2x5.5mL
Ammonia Standard 110 mG/L	1 mL	1 mL	5 mL

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 AND STORAGE

Trigger Enzyme reagent and Standards are ready to use; store tablets tightly sealed with the desiccant pack provided. Kit contents are stable through the labeled expiration date when stored at 2-8 °C.

ASSAY PREPARATION MANUAL TESTING

Working Reagent (For automation refer to Appendix):
Prepare sufficient Working Reagent just prior to testing – 2mL/cuvette; dissolve each Reagent Tablet in 5 mL deionized (DI) water using clean glassware. Mix by gentle inversion. Reconstituted reagent is stable for 1 day refrigerated, allow it to reach room temperature prior to assay.

TESTING PROCEDURE

Pipet each solution into cuvettes, as shown:

	Blank	Standard	Sample(s)
1. **D.I. Water**	**40µL**		
2. **Standard/Sample**		**40µL**	**40µL**
3. **Working Reagent**	**2.0mL***	**2.0mL**	**2.0mL**

Mix cuvettes, incubate 3 minutes.

Zero Spectrophotometer using air or D.I. Water

(NOT Reagent Blank Cuvette**)

Read $A_{INITIAL}$ (Initial ABS)

** Note: 2.0mL = 2000µL*

*** The initial AB S is high; as AMM reacts ABS decreases.*

4. **Trigger Enzyme**	**40µL**	**40µL**	**40µL**

Mix cuvettes, incubate 15-20 minutes, Read A_{FINAL} (Final ABS).

The testing range is up to 170 mG/L. If test result is over-range, dilute the sample with deionized (or distilled) water; re-assay & multiply this test result by the dilution factor.

CALCULATIONS

The Ammonia mg/L concentration may be calculated as below:

1. Our online "Flex Calculator™-AMM" spreadsheet at *http://unitechscientific.com/calculators.htm* is available for download. AMM mG/L values will be calculated automatically. Refer to APPENDIX for **AMM-Nitrogen** calculation.
2. Manual Calculation:

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

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

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

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

Calculate AMM Concentration (based on 110 mG/L Standard)

$$\text{AMM mG/L} = 110 \times \text{dilution factor} \times \frac{\text{Net } A_{SAMPLE}}{\text{Net } A_{STANDARD}}$$

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 AMM** CALCULATIONS:

a. Extinction Coefficient (results based on factor; compare standard result with known value to verify recovery.)

Ammonia (mg/L) = Net A x 142

Factor is derived as follows:

$$\text{Ammonia (mg/L)} = \frac{\text{Net A} \times \text{MW} \times \text{TV}}{(\varepsilon)\,(P)\,(SV)}$$

$$= \frac{\text{Net A} \times 17 \times 2.075}{6.22 \times 1 \times 0.04}$$

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Where: MW = 17 g/mole, molecular wt of Amm
TV = 2.075 mL total reaction volume
SV = 0.04 mL sample volume
ε (absorptivity)= 6.22 at 340 nm
P = 1 cm light path

Adjust calculations if alternate SV and TV are used. Sample volume inaccuracy will affect results with the extinction coefficient calculation method; use calibrated micropipettes.

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

NOTES – **NITROGEN** CALCULATIONS:

1. The total Ammonia Nitrogen plus Primary Amino Nitrogen comprise YANC (Yeast Assimilable Nitrogen Compounds). Calculate AMM-Nitrogen content:

   AMM-Nitrogen content (mg/L) = 82.4% x AMM (mg/L).

2. Determine Primary Amino Nitrogen using Unitech 'PAN' Reagent.

METHODOLOGY & CHEMICAL PRINCIPLES

This Ammonia method is based on that developed by Talke and Schubert.[1] The reaction sequence is as follows:

$$NH_3 + \text{ak-G} + NADH + H^+ \xrightarrow{GLDH} \text{Glutamate} + NAD^+ + H_20$$

Glutamate dehydrogenase (GLDH) catalyzes the condensation of ammonia and alpha ketoglutarate (ak-G) with the concomitant oxidation of reduced nicotinamide adenine dinucleotide (NADH). The oxidation of NADH causes a decrease in absorbance at 340 nm, which is proportional to the amount of ammonia in the sample.

SIGNIFICANCE OF MEASUREMENTS

The content of Ammonia in vinifera grape juice ranges from 15 - 310 mg/L,. The addition of ammonia salts (up to 300 mg/L) to musts has been recommended as nutrients for fermentation yeasts and lactic bacteria. AMM contains 82.4% Nitrogen. Together Ammonia Nitrogen plus Primary Amino Nitrogen comprise YANC (Yeast Assimilable Nitrogen Compounds). Measure Primary Amino Nitrogen using Unitech's PAN Reagent.

AUTOMATED TESTING

'ChemWell for Wine' - Prepare Working Reagent as follows:

	25T	**70T**
Ammonia Reagent Tablets	2	4
Deionized Water	10mL	20mL

(# of Tests accounts for Reagent Bottle dead volume)
Place the Working Reagent and Trigger Enzyme 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.

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