

UNITAB™ REAGENT

AMMONIA CW-EXTENDED RANGE

Enzymatic, UV-Method *Instrumentation must read ABS to 3.5 ABS*

Product #:
AMM2-60 (30 Tests)
AMM2-150 (75 Tests)
AMM2-500 (250 Tests)

INTENDED USE

Ammonia CW-Extended Range UniTAB™ Reagent is intended for measuring ammonia in juice and wine **over a broad measuring range**. Compared to Unitech Scientific's traditional AMMONIA reagent kit, **AMM CW-Extended Range** reagent offers the same sensitive but twice the measuring range (up to **350mg/L**).

KIT CONTENTS

	<u>30T</u>	<u>75T</u>	<u>250T</u>
Ammonia Reagent Tablets	2X12	2X30	2X100
GLDH Trigger Enzyme	1.3 mL	3.3 mL	2x5.5mL
Ammonia Standard 110 mg/L	1 mL	1 mL	5 mL

SYSTEM REQUIREMENT

ChemWell for Wine, or other instrument capable of reading **340 nm ABS over 0 - 3.5 ABS Range** with a 1 cm light path. Standard spectrophotometers may not provide this linearity. (If your instrument is linear in a standard ABS range (0 – 2,0 ABS), contact Unitech Technical Service for our traditional AMM procedure.

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.

AUTOMATED TESTING

Prepare Working Reagent, 'ChemWell for Wine'

Prepare Extended Range Working Reagent by dissolving **two (2)** Reagent Tablet in each 5 mL deionized (DI) water just prior to assay:

	<u>25T</u>	<u>70T</u>
Ammonia Reagent Tablets	4	8
Deionized Water	<u>10mL</u>	<u>20mL</u>

(# of Tests accounts for Reagent Bottle dead volume)

Place 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.

NITROGEN CALCULATIONS: Total YAN values are calculated automatically when using 'ChemWell for Wine' YAN Index. Contact Unitech Technical Service for details.

APPENDIX

Manual Method - using instrument capable of reading **340 nm ABS over 0 - 3.5 ABS Range** with 1 cm light path.

Prepare Working Reagent, manual method:

Prepare Extended Range Working Reagent by dissolving **two (2)** Reagent Tablet in each 5 mL deionized (DI) water just prior to assay:

	<u>5T</u>	<u>10T</u>	<u>20T</u>
Ammonia Reagent Tablets	4	8	16
Deionized Water	10mL	20mL	40mL

TESTING PROCEDURE Manual Method

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 ABS is high; as AMM reacts ABS decreases.

4. Trigger Enzyme	40µL	40µL	40µL
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Mix cuvettes, incubate 15-20 minutes, Read A_{FINAL} (Final ABS).

The testing range is up to 350 mg/L. If a test result is over-range, dilute the sample with deionized (or distilled) water; re-assay & multiply this test result by the dilution factor.

CALCULATIONS Manual Method

Calculate the Ammonia mg/L concentration:

- Our online "Flex Calculator™-AMM" spreadsheet at <http://unitechscientific.com/calculators.htm> is available

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for download. AMM mG/L values will be calculated automatically. For **AMM-Nitrogen**, refer to APPENDIX.

2. Manual Calculation:

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

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

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

$$\text{AMM mG/L} = (110) \times (\text{d.f.}) \times \frac{\text{Net A}_{\text{SAMPLE}}}{\text{Net A}_{\text{STANDARD}}}$$

NITROGEN CALCULATIONS:

1. Calculate **Ammonia Nitrogen** content:

$$\text{AMM-Nitrogen content (mg/L)} = 82.4\% \times \text{AMM (mg/L)}.$$

2. The YANC (Yeast Assimilable Nitrogen Compounds) is calculated as the total AMM-Nitrogen plus Primary Amino Nitrogen comprise. Determine Primary Amino Nitrogen using Unitech 'PAN' Reagent.

QUALITY CONTROL

Test a known Sample (e.g. standard or check wine) in each assay to monitor assay results; 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.)

NOTES FOR ALTERNATE AMM CALCULATIONS:

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

$$\text{Ammonia (mg/L)} = \text{Net A} \times 142$$

Factor is derived as follows:

$$\begin{aligned} \text{Ammonia (mg/L)} &= \frac{\text{Net A} \times \text{MW} \times \text{TV}}{(\epsilon) (P) (SV)} \\ &= \frac{\text{Net A} \times 17 \times 2.075}{6.22 \times 1 \times 0.04} \end{aligned}$$

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.

TRADEMARKS:

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

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