

# AMMONIA

*Enzymatic UV-Method*

Product #

AMM-30 (60 Tests)

AMM-150 (75 Tests) AMM-500 (250 Tests)

### INTENDED USE

Unitech Scientific Ammonia Reagent is intended for the determination of ammonia in wine and other liquid samples.

### METHODOLOGY & CHEMICAL PRINCIPLES

This Ammonia method is based on that developed by Talke and Schubert.<sup>1</sup> The reaction sequence is as follows:



$\text{NH}_3 + \text{ak-G} + \text{NADH} + \text{H}^+ \text{-----} > \text{Glutamate} + \text{NAD}^+ + \text{H}_2\text{O}$   
 Glutamate dehydrogenase (GIDH) 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.

### REAGENTS

Active Ingredients are:

	Concentration	Quantity/Kit		
	as Formulated	60T	75T	250T
<u>Ammonia Reagent Tablets</u>		24	60	200
Alpha Ketoglutarate	1.87 mmol/L			
Adenosine Diphosphate	1.60 mmol/L			
NADH (yeast)	0.35 mmol/L			
Buffered at pH 7.6				
<u>Trigger Enzyme</u>		1.2	3.5	11.6mL
Glutamate Dehydrogenase	15 kU/L			
<u>Ammonia Standard</u>	110 mg/L	1mL	1mL	5mL

### STORAGE & REAGENT PREPARATION

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. Trigger Reagent and Standard are ready to use and do not require reconstitution.

### Working Reagent (WRgt):

Prepare sufficient WRgt for all samples and standards in the assay using clean glassware; mix by gentle inversion.

Examples are provided in the table below.

MANUAL TESTING	5 Tests	10 Tests	20 Tests
Ammonia Tablets	2	4	8
Deionized Water	10mL	20mL	40mL

### CHEMWELL (AUTOMATED) 25 Tests 70 Tests 160 Tests

Ammonia Tablets	2	4	8
Deionized Water	10mL	20mL	40mL

(# of Tests accounts for Reagent Bottle dead volume)

The WRgt is stable for 7 days at 2-8°C. Discard any turbid reagent or reagent with an absorbance less than 1.20 at 340 nm read against the DI water blank.

### PROCEDURE

System parameters: Wavelength 340 nm, Absorbance Range 0-2.5A at 1cm pathlength. Refer to NOTES for alternative sample volumes (SV), measuring range, etc.

1. Label one cuvette for each sample, standard and blank.
2. Pipet standard, samples, and water into cuvettes as shown below, using calibrated micropipettes.

Pipet into Cuvettes	Reagent Blank Cuvette	Sample or Standard Cuvette
<b>Sample</b>		40 µL
<b>DI water</b>	40 µL	
<b>Working Reagent</b>	2 mL	2 mL
Mix cuvettes and wait 3 minutes. Zero spectrophotometer (water). Read A <sub>INITIAL</sub> (Initial absorbance).		
<b>Trigger Enzyme</b>	35 µL (1 drop)	35 µL (1 drop)
Mix and incubate 10-20 min. Read A <sub>FINAL</sub> (Final absorbance).		

3. Dispense WRgt into each cuvette, mix and wait 3 minutes. Zero spectrophotometer with deionized water. Read initial absorbance (A<sub>INITIAL</sub>) values.
4. Gently mix the Trigger Enzyme reagent by inversion and dispense, as shown above. Mix each cuvette, wait 10-20 minutes and read the final absorbance (A<sub>FINAL</sub>).

### CALCULATIONS

1. Calculate delta A = A<sub>INITIAL</sub> - A<sub>FINAL</sub> for each cuvette.
2. If the delta A of the Reagent Blank (A<sub>I</sub> - A<sub>F</sub>) is significant, subtract this value from the delta A of each sample and standard.  

$$\text{Net } A_{\text{SAMPLE}} = \text{delta } A_{\text{SAMPLE}} - \text{delta } A_{\text{BLANK}}$$
3. Samples with delta A values less than 0.05 should be reassayed with a larger sample volume.
4. Select one of the following calculation methods:

$$\begin{aligned} \text{a. Extinction Coefficient} \\ \text{Ammonia (mg/L)} &= \frac{\text{Net } A \times \text{MW} \times \text{TV}}{(\epsilon) (P) (SV)} \\ &= \frac{\text{Net } A \times 17 \times 2.075}{6.22 \times 1 \times 0.04} = \text{Net } A \times 142 \end{aligned}$$

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 or if diluted samples are assayed.

- b. A Single-point Standard, e.g. 110 mg/L Ammonia

$$\text{Ammonia, mg/L} = \text{Conc. Standard} \times \frac{\text{Net } A_{\text{SAMPLE}}}{\text{Net } A_{\text{STANDARD}}}$$

$$\text{Ammonia, mg/L} = 110 \times \frac{\text{Net } A_{\text{SAMPLE}}}{\text{Net } A_{\text{STANDARD}}}$$

- c. A Multi-point Standard (e.g. Unitech 5-Point Standards): Sample concentrations are calculated from the best-fit standard curve.

## NOTES

1. Verify spectrophotometer linearity at 340nm by reading working reagent neat and at 1:2 (1mL+1mL DI water.) If neat result is not twice diluted result, dissolve 2 tablets in 7 mL DI water.
2. Wavelength: NADH absorbance maximum is 340 nm. Therefore, 334-340 nm determinations provide the best analytical discrimination. While less sensitive, 365 nm analysis provides approximately 1.5-fold broader measuring range.
3. Linearity and Sample Dilution: Most samples may be run neat; those higher than 225 mg/L (or produce a final absorbance <0.10) should be diluted with deionized water and reassayed:

<u>Estimated Ammonia</u>	<u>Dilution</u>
≥ 2 g/L	1:100
225 mg/L to 2 g/L	1:10
< 225 mg/L	neat

Multiply the calculated g/L result by the dilution factor. For example, when diluting 1 part sample with 9 parts water, the dilution factor is "10".

4. Creep reactions occasionally occur as a result of enzymes or pigments in the sample interfering with the enzymatic reactions. If necessary, prepare a sample blank, i.e. prepare two tubes:
  - a. Sample Blank [Rgt + Sample]
  - b. Reaction [Rgt + Sample + Trigger]
 Corrected delta A = delta A<sub>REACTION</sub> - delta A<sub>BLANK</sub>.  
Use this corrected delta A to calculate results.
5. Calculate Ammonia nitrogen as follows:  
'Ammonia nitrogen' mg/L = 82.4% x Ammonia mg/L.

## SAMPLE PREPARATION

### Clarification

Turbid samples should be filtered. Fermentation samples may be clarified by centrifugation (if necessary) and placed into a water bath at 80°C to inactivate fermentation enzymes.

### Decolorization

Red wine typically needs decolorization only when SV larger than 100 µL are used. If an unacceptably high sample blank absorbance is obtained, mix 10 mL juice and approximately 0.1 g polyamide powder or polyvinylpoly-pyrrolidone (PVPP), stir for 1 minute and filter.

## SIGNIFICANCE OF MEASUREMENT

The content of Ammonia in vinifera2 grape juice ranges from 15 - 310 mg/L, (0 - 160 mg/L for other fruit juices).<sup>3</sup> The addition of ammonia salts (up to 300 mg/L) to musts has been recommended as nutrients for fermentation yeasts and lactic bacteria.<sup>4,5,6,7</sup>

Together Ammonia Nitrogen\* plus Primary Amino Nitrogen comprise YANC (Yeast Assimilable Nitrogen Compounds). Calculate Ammonia nitrogen as per Note 5 above.

Determine primary amino nitrogen using Unitech Reagent # PAN.

## QUALITY CONTROL

It is recommended that low and high controls be included in each set of assays. Factors that may affect the performance of this test include proper instrument function, temperature, cleanliness of glassware and pipetting accuracy. Linearity controls are available from Unitech or GENE-TRAK Systems.

## REFERENCES

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