

AMMONIA (NH₃)

Enzymatic UV – Method
RX MONZA
FOOD AND WINE

INTENDED USE

For the quantitative *in vitro* determination of Ammonia in food and wine. This product is suitable for use on the RX **monza** analyser.

FOR THE ANALYSIS OF FOOD AND WINE. Not for diagnostic procedures.

Cat. No.

AM 1015	R1a. Reagent	10 x 5 ml
10 x 5 ml	R1b. Buffer	70 ml
	R2. GLDH	1 x 1 ml
	CAL Standard	5.5 ml

SIGNIFICANCE

Before fermentation can begin the status of the raw material must be evaluated to see if supplementation is required. This includes the estimation of yeast available nitrogen (YAN) a vital component required in fermentation. If levels of YAN are too low this can result in sluggish or stuck fermentation compromising the quality of the product. The most important inorganic source of YAN is ammonia, testing for ammonia ions is an important way of monitoring nitrogen levels and the fermentation process.

PRINCIPLE^(1,2,3)



Ammonia combines with α -ketoglutarate and NADPH in the presence of glutamate dehydrogenase (GLDH) to yield glutamate and NADP⁺. The corresponding decrease in absorbance at 340 nm is proportional to the ammonia concentration in the sample.

REAGENT COMPOSITION

Contents	Initial Concentration of Solutions
R1a. Reagent	
NADPH	0.26 mmol/l
α -ketoglutarate	3.88 mmol/l
R1b. Buffer	
Triethanolamine	0.15 mol/l, pH 8.6
R2. GLDH	≥ 1200 U/ml
CAL Standard	See lot specific insert

SAMPLE

Red wine, white wine and fruit juices. Turbid samples should be filtered prior to assay. Strongly coloured samples with suspected low values should be decolourised with 0.1g polyamide powder, gelatine or polyvinylpyrrolidone (PVPP) to approximately 10 ml of juice or wine. Stir for 1 minute and filter. The clear filtrate can then be used in the assay undiluted.

Samples with ammonia concentration <20 mg/l can be run neat. If ammonia concentration is >20 mg/l, samples should be pre-diluted with deionised water prior to assay.

Dilution Table

Estimated concentration of ammonia (mg/L)	Dilution with water	Dilution Factor (F)
<20	No dilution required	1
20-200	1 + 9	10
200-2000	1 + 99	100

SAFETY PRECAUTIONS AND WARNINGS

For the analysis of food and wine. Not for diagnostic procedures. Do not pipette by mouth. Exercise the normal precautions required for handling laboratory reagents.

Solutions R1b, R2 and CAL contain Sodium Azide. Avoid ingestion or contact with skin or mucous membranes. In case of skin contact, flush affected area with copious amounts of water. In case of contact with eyes or if ingested, seek immediate medical attention.

Sodium Azide reacts with lead and copper plumbing, to form potentially explosive azides. When disposing of such reagents flush with large volumes of water to prevent azide build up. Exposed metal surfaces should be cleaned with 10% sodium hydroxide.

Health and Safety data sheets are available on request.

Please dispose of all Biological and Chemical materials according to local guidelines.

The reagents must be used only for the purpose intended by suitably qualified laboratory personnel, under appropriate laboratory conditions.

STABILITY AND PREPARATION OF REAGENTS

R1a. Reagent

Reconstitute the contents of one vial R1a with 5 ml Buffer R1b. Stable for 5 days at +15 to +25°C or 3 weeks at +2 to +8°C, in the absence of bacterial contamination.

R1b. Buffer

Contents ready for use. Stable up to the expiry date specified when stored at +2 to +8°C.

R2. GLDH

Contents ready for use. Stable up to the expiry date specified when stored at +2 to +8°C

CAL Standard

Contents ready for use. Stable up to the expiry date when stored at +2 to +8°C.

RI = Reagent/Buffer

R2 = GLDH

MATERIALS PROVIDED

Reagent
 Buffer
 GLDH
 Standard

MATERIALS REQUIRED BUT NOT PROVIDED

Randox Ammonia Ethanol Controls:

Level 2 (Cat. No. EA 1367)

Level 3 (Cat. No. EA 1368)

PROCEDURE

Select NH3-WINE in the Run Test screen and carry out a water blank as instructed.

Pipette into cuvette:

	Reagent Blank	Standard	Sample
Sample	---	---	70 µl
Distilled water	70 µl	---	---
Standard	---	70 µl	---
Reagent (R1)	700 µl	700 µl	700 µl

Mix, and incubate for 5 min at +37°C. Insert the cuvette into the RX Monza flowcell holder when prompted for Sample Blank and press Read. Then add

GLDH (R2)	7 µl	7 µl	7 µl
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Mix, incubate for a further 5 min at +37°C. Insert the cuvette into the Rx Monza flowcell holder when prompted for Sample and press Read.

CALIBRATION FOR RX MONZA

Calibration is recommended with change in reagent lot or as indicated by quality control procedures. Use CAL Standard provided in the kit.

QUALITY CONTROL

Randox Ammonia Ethanol Controls Level 2 and Level 3 are recommended for daily quality control. Two levels of controls should be assayed at least once a day. Values obtained should fall within a specified range. If these values fall outside the range and repetition excludes error, the following steps should be taken:

1. Check instrument settings and light source.
2. Check cleanliness of all equipment in use.
3. Check water, contaminants i.e. bacterial growth may contribute to inaccurate results.
4. Check reaction temperature.
5. Check expiry date of kit and contents.
6. Contact Randox Laboratories Customer Technical Support, Northern Ireland +44 (0) 28 9442 2413.

SPECIFIC PERFORMANCE CHARACTERISTICS

The following Ammonia performance characteristics were obtained using a RX Monza analyser in cuvette mode at +37°C.

LINEARITY

The method is linear to ammonia concentration of 20 mg/l. Samples above this concentration should be pre-diluted according to the dilution table.

SENSITIVITY

The minimal detectable concentration of ammonia with an acceptable level of precision was determined as 1 mg/l.

PRECISION

Within Run precision

	Level 2	Level 3	Grape juice
Mean (mg/l)	2.94	5.48	105.2
S.D	0.117	0.138	0.186
C.V.(%)	3.97	2.51	1.77
n	20	20	20

Total Run precision

	Level 2	Level 3	Grape juice
Mean (mg/l)	2.83	5.48	105.2
S.D	0.155	0.136	0.231
C.V.(%)	5.46	2.48	2.20
n	20	20	20

CORRELATION

The Randox method (Y) was compared to another commercially available method (X). Linear regression analysis of the data resulted in the following equation:

$$Y = 1.007X + 0.4283$$

and a correlation coefficient $r = 0.9928$

50 samples were analysed spanning the range 4.15 to 110.9 mg/l.

REFERENCES

1. Dewan, J.G., Biochem J., 1938; **32**: 1378.
2. Neely, W.E., Phillipson, J., Clin Chem, 1988; **34**:1868.
3. Pesh-Iman, M., Kumar, S., Willis, C.E., Clin. Chem., 1978; **24**:2044.