RANDOX

PRIMARY AMINO NITROGEN (NOPA)

Colorimetric - Method
RX ALTONA
MANUAL
FOOD AND WINE

INTENDED USE

For the quantitative determination of Primary Amino Nitrogen in food and wine. This product is suitable for manual use and on the RX **altona** analyser. **Applications for a variety of additional analysers are available from www.randoxfooddiagnostics.com.**

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

Cat. No.			
NO 3495	R1a.	Buffer	1 x 104 ml
	R1b.	NAC	5 x 20 ml
	R2.	OPA	1 x 12 ml
	CAL	Standard	1 x 5 ml

SIGNIFICANCE

The 2 main sources of nitrogen for yeast are ammonia and primary amino acids. As nitrogen is the limiting element in the growth of yeast cells it is important to measure the concentration of Yeast Assimilable Nitrogen (YAN). Limitation of YAN has been identified as the main cause of 'stuck' fermentation, while high levels in the presence of ethanol can lead to formation of potential carcinogens especially where levels of L-arginine are present.

PRINCIPLE

Primary amino nitrogen groups available from primary amino acids react with N-acetyl-L-cysteine (NAC) and o-phthaldialdehyde (OPA) to form isoindoles. The isoindole derivative formed in this reaction can be measured by an increase in absorbance at 340 nm and is stoichiometric with the amount of free primary amino nitrogen present in the sample.

SAMPLE

Red wine, white wine and fruit juices. Turbid samples should be filtered prior to assay.

SAFETY PRECAUTIONS AND WARNINGS

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

Solutions R1 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. Buffer
Contents ready for use. Stable up to the expiry date specified when stored at +2 to +8°C.

R1b. NAC
Reconstitute the contents of one vial of R1b with 20 ml of R1a. Stable for 21days at +2 to +8°C or 10 days at +15 to +25°C.

R2. OPA
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 specified when stored at +2 to +8°C.

MATERIALS PROVIDED

Buffer
NAC
OPA
Standard

MATERIALS REQUIRED BUT NOT PROVIDED

Double deionised / distilled water

RX ALTONA PROCEDURE

Select NOPA in the Test Screen. Then select Run Calibration or Run Sample and carry out a water blank as instructed.

Pipette into cuvette:

	Reagent Blank S0	Standard S1	Sample
ddH2O	8 µl	---	---
Standard (S1)	---	8 µl	---
Sample	---	---	8 µl
Buffer/NAC (R1)	800 µl	800 µl	800 µl

Mix, and incubate for 2 minutes at +25°C. Insert the cuvette into the RX **altona** flowcell holder when prompted for Sample Blank and press Read. Then add

OPA (R2)	80 µl	80 µl	80 µl

Mix, and incubate for 5 minutes at +25°C. Insert the cuvette into the RX **altona** flowcell holder when prompted for Sample and press Read.

CALIBRATION FOR RX ALTONA

A 2 point linear calibration is recommended with change in reagent lot or as indicated by quality control procedures. Use CAL Standard provided in the kit.

FOR MANUAL USE
PROCEDURE SEMI MICRO

Wavelength:	340 nm
Cuvette:	1 cm path length
Temperature:	+20 to +25°C
Measurements:	Against Reagent Blank

Pipette into 1 ml cuvette

	Blank	Standard	Sample
Buffer/NAC (R1)	1000 µl	1000 µl	1000 µl
ddIH2O	10 µl	-	-
Standard	-	10 µl	-
Sample	-	-	10 µl

Mix, and read absorbance A_1 after 2 minutes.

OPA (R2)	100 µl	100 µl	100 µl

Mix and read absorbance A_2 after 5 minutes.

PROCEDURE MACRO

Wavelength:	340 nm
Cuvette:	1 cm path length
Temperature:	+20 to+25°C
Measurements:	Against Reagent Blank

Pipette into cuvette

	Blank	Standard	Sample
Buffer/NAC (R1)	2000 µl	2000 µl	2000 µl
ddIH2O	20 µl	-	-
Standard	-	20 µl	-
Sample	-	-	20 µl

Mix, and read absorbance A_1 after 2 minutes.

OPA (R2)	200 µl	200 µl	200 µl

Mix and read absorbance A_2 after 5 minutes.

MANUAL CALCULATION

Determine absorbance differences $A_2 - A_1$, for blank and sample.

$$\Delta A = \Delta A_{\text{sample}} - \Delta A_{\text{blank}}$$

$$\text{Concentration} = \frac{\Delta A_{\text{sample}}}{\Delta A_{\text{standard}}} \times \text{conc. of standard}$$

SPECIFIC PERFORMANCE CHARACTERISTICS

The following Primary Amino Nitrogen performance characteristics were obtained using a RX **altona** analyser in cuvette mode at +25°C.

LINEARITY

The primary amino nitrogen assay is linear to 500 mg N/l.

SENSITIVITY

The minimal detectable concentration of primary amino nitrogen with an acceptable level of precision was determined as 4.6 mg N/l.

PRECISION

Intra assay precision

	Level 1	Level 2	Level 3
Mean (mg N/l)	67.8	210.2	341.3
S.D	2.754	3.474	5.150
C.V. (%)	4.06	1.65	1.51
n	20	20	20

Inter assay precision

	Level 1	Level 2	Level 3
Mean (mg N/l)	68.6	213.7	348.2
S.D	3.042	7.384	8.329
C.V. (%)	4.44	3.46	2.39
n	20	20	20

27 Sep 16 ml
Rev. 002