ACETIC ACID

UV METHOD
RX MONACO

INTENDED USE

For the quantitative determination of Acetic Acid in wine. This product is suitable for use on the RX **monaco** analyser.

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

Cat. No.

AT 8362	R1a.	Buffer	5 x 20 ml
	R1b.	Substrate	5 x 20 ml
	R1c.	Enzyme reagent 1	1 x 1.1 ml
	R2a.	Enzyme reagent 2	4 x 10 ml
	R2b.	Diluent	4 x 10 ml
	CAL.	Standard	1 x 10 ml

SIGNIFICANCE

Acetic acid is the most commonly occurring volatile acid present in low concentrations in most food and beverages. It is involved in the metabolic processes during the ripening of fruit and is a key indicator of wine quality. During and subsequent to alcohol fermentation a small amount of acetic acid is produced and is thought to contribute to the complexity of the wine production process. In early fermentation analysis monitoring of acetic acid levels is important with high levels indicative of spoilage bacteria such as acetobacter. If this occurs it may result in a dissatisfying taste and smell of the wine.

PRINCIPLE (1, 2, 3)

Acetic acid (acetate) is converted to acetyl - CoA by acetyl CoA synthetase (ACS).

$$\text{Acetate + ATP + CoA} \xrightarrow{\text{ACS}} \text{Acetyl - CoA + AMP +pyrophosphate}$$

Acetyl CoA reacts with oxaloacetate in the presence of citrate synthase (CS) to produce citrate. The oxaloacetate for this reaction is produced from the conversion of malate by L-malate dehydrogenase (L-MDH) with the reduction of NAD to NADH.

$$\text{Acetyl CoA + oxaloacetate + } H_2O \xrightarrow{\text{CS}} \text{citrate + CoA}$$

$$\text{L-malate + NAD}^+ \underset{}{\overset{\text{L-MDH}}{\rightleftharpoons}} \text{oxaloacetate + NADH + H}^+$$

The formation of NADH is measured as an increase in absorbance although this is not directly proportional to the concentration of acetic acid.

SAMPLE

Use clear liquid samples for the assay. Turbid samples should be filtered prior to assay.
Acetic acid can be determined in red wine and white wine without decolourising and sample dilution is only required when Acetic acid concentration is > concentration of the standard supplied with the kit.

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.

Solution R1a contains 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

R1. Buffer / Substrate / Enzyme Reagent 1
Reconstitute 1 vial of Substrate R1b with 1 bottle of Buffer R1a. Stable for 2 weeks at +2 to +8°C. Before use add **200 µl** of Enzyme Reagent 1(R1c). Stable for 1 day at +2 to +8°C.

R2. Enzyme Reagent 2
Reconstitute 1 vial of Enzyme Reagent 2 (R2a) with 1 bottle of Diluent (R2b). Stable for 5 days at +2 to +8°C.

CAL. Acetic Acid Standard
Contents ready for use. Stable up to expiry date when stored at +2 to +8°C.

MATERIALS PROVIDED

Buffer / Substrate / Enzyme Reagent 1
Enzyme Reagent 2 / Diluent
Acetic Acid Standard

MATERIALS REQUIRED BUT NOT PROVIDED

Double deionised water

PROCEDURE NOTES

The Chemistry Parameters for Randox Dedicated RX **series** Assays are predefined on the hard drive of the analyser PC. The required programs should be downloaded to the analyser software. Please note that the predefined chemistry parameters use SI units. If alternative units are required these can be edited by the user. In this case the technical range should be edited in accordance with the users selected units. For wine testing, units and technical range parameters should be entered exactly as they appear on this insert. All necessary instructions are encoded on the reagent barcode. If the barcode can not be read by the analyser, enter manually the series of numbers given beneath the barcode. If problems continue, contact Randox Laboratories Customer Technical Services, Northern Ireland +44 (0) 28 9445 1070.

CALIBRATION

A standard series should be prepared by diluting the standard as detailed on the Lot Specific value sheet.

A 5 point log-logit calibration is recommended daily.

This assay uses a **"Logit-log5P"** calculation.

SPECIFIC PERFORMANCE CHARACTERISTICS

The following Acetic Acid performance characteristics were obtained using a RX **monaco** analyser.

LINEARITY

The method is linear up to the concentration of the standard. In the event of a re-run dilution should be selected and linearity is extended to the concentration of the standard x3.

SENSITIVITY

The minimal detectable concentration of Acetic Acid with an acceptable level of precision was determined as 0.03 g/l.

PRECISION

Intra assay precision

	Level 1	Level 2	Level 3
Mean (g/l)	0.228	0.421	0.783
S.D	0.005	0.008	0.015
C.V (%)	2.31	1.96	1.92
n	20	20	20

Inter assay precision

	Level 1	Level 2	Level 3
Mean (g/l)	0.231	0.414	0.778
S.D	0.004	0.007	0.018
C.V (%)	1.91	1.64	2.28
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 = 0.99X + 0.00
and a correlation coefficient r = 0.99

50 samples were analysed spanning the range 0.072 to 0.930 g/l.

REFERENCE

1. Bergmeyer, H.U. & Möllering, H. (1974) in Methods of Enzymatic Analysis (Bergmeyer, H.U., ed.) 2nd ed., vol.**3**, p.1520-1528, Verlag Chemie, Weinheim, Academic Press, Inc. New York and London.
2. Beutler, H.O. (1984) in Methods of Enzymatic Analysis (Bergmeyer, H.U.,ed.) 3rd ed., vol **VI**, pp. 639-645, Verlag Chemie, Weinhelm, Deerfield Beach/Florida, Basel.
3. Frenkel, E.P. & Kitchens, R.L. (1981) in Methods of Enzymology, **71**, pp. 317-324. Academic Press Inc. New York and London.

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