ACETIC ACID

UV Method
MANUAL

INTENDED USE

For the quantitative *in vitro* determination of Acetic Acid in Red wine, white wine, beer or fruit juices. This product is suitable for Manual use. **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.			
AT 2654	R1a.	Buffer	1 x 50 ml
4 x 10 ml	R1b.	Substrate	4 x 10 ml
	R1c.	Enzyme Reagent 1	1 x 0.5 ml
	R2a.	Enzyme Reagent 2	4 x 0.12 ml
	R2b.	Diluent	2 x 5 ml
	CAL.	Acetic Acid Standard	1 x 5.5 ml

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 COLLECTION AND PREPARATION (4, 5)

Red wine, white wine, beer or fruit juices. Decolourisation of red wine is not necessary. Fruit juices can also be assayed but must be filtered if turbid. Strongly coloured juices or red wine with suspected low values should be decolourised with 0.1 g polyamide powder, gelatine or polyvinylpolypyrrolidone (PVPP) to approximately 10 ml of juice. Stir for 1 minute and filter. The clear filtrate can then be used in the assay undiluted. Carbonic acid should be removed from beer by filtering or stirring with a glass rod for 30 seconds. Adjust acidic samples to pH 8-9 by adding sodium hydroxide solution and incubating for 15 minutes.

REAGENT COMPOSITION

Contents	Concentration in the test
R1a. Buffer/Enzymes	
Pipes	100 mmol/l, pH 7.6
ATP	4 mmol/l
NAD^+	3 mmol/l
Magnesium ions	14 mmol/l
R1b. Substrate	
L-malate	100 mmol/l
Coenzyme	0.2 mmol/l
R1c. Enzyme Reagent 1	
L-MDH	>9 ku/l
Citrate synthase	>2 ku/l
R2a. Enzyme Reagent 2	
Acetyl CoA Synthetase	276 u/l
R2b. Diluent	
Phosphate buffer	54 mmol/l
CAL.Standard	See lot specific insert

SAFETY PRECAUTIONS AND WARNINGS

For *in vitro* use only. 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

R1a. Buffer

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

R1b. Substrate

Reconstitute 1 vial of substrate R1b with 10 ml of buffer R1a. Stable for 2 weeks when stored at +2 to +8°C or 4 days at +15 to +25°C.

R1c. Enzyme Reagent 1

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

R2a. Enzyme Reagent 2

Carefully open 1 vial of enzyme reagent R2a and reconstitute with 0.12 ml of diluent R2b. Stable for 7 days at +2 to +8°C or 3 days at +15 to +25°C.

R2b. Diluent

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

CAL. Acetic Acid Standard

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

MATERIALS PROVIDED

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

PROCEDURE SEMI MICRO

Wavelength	340 nm (Hg 334 nm or Hg 365 nm)
Cuvette	1 cm path length
Temperature	+20 to +25°C
Measurements	against water (increasing absorbance)

Pipette into 1 ml cuvettes

	Blank	Standard	Sample
Buffer/Substrate R1	1000 µl	1000 µl	1000 µl
Sample	-	-	30 µl
Standard (optional)	-	30 µl	-
Distilled water	30 µl	-	-

mix and measure absorbance A_o

Enzyme Reagent 1 (R1c)	10 µl	10 µl	10 µl

Mix and incubate at +20 to +25°C for approximately 3 minutes. Measure absorbance A_1

Enzyme Reagent 2 (R2a)	10 µl	10 µl	10 µl

Mix and incubate at +20 to +25°C for 10 - 15 minutes. Measure absorbance A_2

PROCEDURE MACRO

Wavelength	340 nm (Hg 334 nm or Hg 365 nm)
Cuvette	1 cm path length
Temperature	+20 to +25°C
Measurements	against water (increasing absorbance)

Pipette into cuvettes

	Blank	Standard	Sample
Buffer/Substrate R1	2000 µl	2000 µl	2000 µl
Sample	-	-	60 µl
Standard (optional)	-	60 µl	-
Distilled water	60 µl	-	-

mix and measure absorbance A_o

Enzyme Reagent 1 (R1c)	20 µl	20 µl	20 µl

Mix and incubate at +20 to +25°C for approximately 3 minutes. Measure absorbance A_1

Enzyme Reagent 2 (R2a)	20 µl	20 µ l	20 µl

Mix and incubate at +20 to +25°C for 10 - 15 minutes. Measure absorbance A_2

CALCULATION

Determine the absorbance differences (A_1 - A_0) and (A_2 - A_o) for the blank and the sample.

As the measured absorbance is not directly proportional to the acetic acid concentration, the following formula should be used to calculate the $\Delta A_{\text{acetic acid}}$.

$$\Delta A_{\text{acetic acid}} = [(A_2-A_0)_{\text{sample}} - \frac{(A_1-A_0)^2_{\text{sample}}}{(A_2-A_0)_{\text{sample}}}] - [(A_2-A_0)_{\text{blank}} - \frac{(A_1-A_0)^2_{\text{blank}}}{(A_2-A_0)_{\text{blank}}}]$$

Wavelength	Conc. Acetic Acid g/l
Hg 334 nm	0.340 x $\Delta A_{\text{acetic acid}}$
Hg 340 nm	0.334 x $\Delta A_{\text{acetic acid}}$
Hg 365 nm	0.618 x $\Delta A_{\text{acetic acid}}$

OPTIONAL CALCULATION

$$\text{Concentration of acetic acid} = \text{standard conc.} \times \frac{\Delta A_{\text{sample}}}{\Delta A_{\text{standard}}} \text{ (g/l)}$$

LINEARITY

The test is linear to an acetic acid concentration of 0.3 g/l. Dilute samples above this concentration 1 + 9 with distilled/deionised water and multiply the result by 10.

REFERENCE

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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.
4. Junge, Ch. & Spadinger, Ch. (1979) Die flüchtigen Säuren des Weines, Deutsche Lebensmittel-Rundschau. **75**, 12-15.
5. Klopper, W.J., Angelino, S.A.G.F., Tuning, B. & Vermiere, H.A. (1986) Organic acids and glycerol in beer, J. Inst. Brew. **92**, 225-228.

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