L-MALIC-ACID

UV METHOD
MANUAL
RX MONZA

INTENDED USE

For the quantitative *in vitro* determination of L-Malic-Acid in red and white wine, beer and fruit juices. This product is suitable for manual use and on the RX **monza** 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.		
ML 2634	R1a. Buffer/Substrate	1 x 50 ml
4 x 10 ml	R1b. Enzyme/Coenzyme	4 x 10 ml
	R2. Starter	1 x 1 ml
	CAL. Standard	1 x 5 ml

UV METHOD[1,2]

L-malic acid (L-malate) is determined according to the following reaction.

$$\text{L-malate} + NAD^+ \xrightarrow{\text{(L-MDH)}} \text{oxaloacetate} + NADH + H^+$$

$$\text{Oxaloacetate} + \text{L-glutamate} \xrightarrow{\text{AST}} \text{L-aspartate} + \text{2-oxoglutarate}$$

SAMPLE[3]

Use clear liquid samples for the assay. Turbid samples should be filtered prior to assay.
L-malic acid can be determined in red wine, white wine, beer or fruit juices without decolourising or sample dilution.

REAGENT COMPOSITION

Contents	Initial Concentrations
R1a. Buffer/Substrate	
Tris	200 mmol/l, pH 9.0
Glycylglycine	200 mmol/l
L-glutamic acid	50 mmol/l
R1b. Enzyme/Coenzyme	
NAD	6 mmol/l
Aspartate Amino-transferase	≥ 1.8 KU/l
R2. Starter	
Tris	100 mmol/l, pH 7.6
Malate dehydrogenase	2.7 KU/ml
CAL. Standard	
L-Malic Acid	See lot specific insert

SAFETY PRECAUTIONS AND WARNINGS

Do not pipette by mouth. Exercise the normal precautions required for handling laboratory reagents.

Solutions R1a and R2 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/Substrate
Contents ready for use. Stable up to expiry date when stored at +2 to +8°C

R1b. Enzyme/Coenzyme
Reconstitute the contents of one vial of Enzyme/Coenzyme R1b with **1 ml** of deionised/distilled water. Stable for 3 weeks at +2°C to +8°C or 1 week at +15°C to +25°C.

R2. Starter
Contents ready for use as supplied. Stable up to expiry date when stored at +2°C to +8°C in the absence of contamination.

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

STABILITY AND PREPARATION OF WORKING REAGENT (R1)

Enzyme/Coenzyme R1b	Buffer/Substrate R1a
1 ml	9 ml
0.5 ml	4.5 ml

Stable for 8 hours at +2°C to +8°C.

MATERIALS PROVIDED

Buffer/Substrate
Enzyme/Coenzyme
Starter
Standard

MATERIALS REQUIRED BUT NOT PROVIDED

Double deionised/ distilled water

PROCEDURE NOTES

If total L-malate content (the sum of free and esterfied L-malate) is required, heat 20 ml of wine and 6 ml sodium hydroxide (2 mol/l) for 30 mins under a reflux condenser while stirring. Allow the sample to cool (+2°C to +8°C) and neutralise (pH 7.0) with sulphuric acid (1 mol/l). Transfer to a 50 ml volumetric flask and add water to the 50 ml mark. After filtration the clear solution can be used in the assay. Carbonic acid can be removed from beer by stirring the sample with a glass rod for 1 minute.

PROCEDURE

Select MA in the Run Test screen and carry out a water blank as instructed.

Pipette into a cuvette:

	Reagent Blank S0	Standard S1	Sample
Redist. Water	10 µl	-	-
Standard	-	10 µl	-
Sample	-	-	10 µl
Working Rgt R1	500 µl	500 µl	500 µl

Mix, incubate for 3 minutes at +20 to+25°C or +37°C. Insert the cuvette into the RX **monza** flowcell holder when prompted for Sample Blank and press Read. Then add

Starter R2	5 µl	5 µl	5 µl

Mix, incubate for a further 5 minutes at +20 to+25°C or +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.

FOR MANUAL USE
PROCEDURE SEMI MICRO

Wavelength:	340 nm (Hg 334 nm or Hg 365 nm)
Cuvette:	1 cm path lengh
Temperature:	+20 to+25/+37°C
Measurements:	against water (increasing absorbance)

Prepare Working Reagent and pipette into 1 ml cuvettes

	Blank	Standard	Sample
Working Reagent (R1)	1000 µl	1000 µl	1000 µl
Redist. Water	20 µl	-	-
Sample	-	-	20 µl
Standard	-	20 µl	-

Mix and read absorbance A_1 after approximately 3 minutes.

Starter 3	10 µl	10 µl	10 µl

Mix and read absorbance A_2 after approximately 5-10 minutes.

PROCEDURE MACRO

Wavelength:	340 nm (Hg 334 nm or Hg 365 nm)
Cuvette:	1 cm path lengh
Temperature:	+20 to+25/+37°C
Measurements:	against water (increasing absorbance)

Prepare Working Reagent and pipette into cuvettes

	Blank	Standard	Sample
Working Reagent (R1)	2000 µl	2000 µl	2000 µl
Redist. Water	40 µl	-	-
Sample	-	-	40 µl
Standard	-	40 µl	-

Mix and read absorbance A_1 after approximately 3 minutes.

Starter 3	20 µl	20 µl	20 µl

Mix and read absorbance A_2 after approximately 5-10 minutes.

MANUAL CALCULATION

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

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

To calculate the L-Malic Acid concentration use the following formula:

$$\text{g/l L-Malic Acid} = 1.096 \times \Delta A$$

Alternatively,

$$\text{g/l L-Malic Acid} = \text{standard conc.} \times \frac{\Delta A_{\text{sample}}}{\Delta A_{\text{standard}}}$$

SPECIFIC PERFORMANCE CHARACTERISTICS

The following performance data were obtained using a RX **monza** analyzer in cuvette mode at +37°C.

LINEARITY

The test is linear to L-malic acid concentration of 1.43 g/l. Dilute sample above this concentration 1+9 with distilled water. Multiply the result by 10.

SENSITIVITY

The minimum detectable concentration of L-malic acid with an acceptable level of precision was determined as 0.136 g/l.

PRECISION

Within run precision

	Level 1	Level 2
Mean	0.229	0.675
SD	0.007	0.011
CV(%)	3.09	1.58
n	20	20

Between run precision

	Level 1	Level 2
Mean	0.229	0.675
SD	0.008	0.011
CV(%)	3.44	1.56
n	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 = 0.9037X + 0.0514
and a correlation coefficient r = 0.9828

40 patient samples were analyzed spanning the range 0.14 to 0.99 g/l.

REFERENCES

1. Mollering, H. (1974) in Methods of Enzymatic Analysis (Bergmeyer, H.K., ed.) 2nd ed., vol 3 pp 1589 - 1593, Verlag Chemie,Weinheim, Academic Press, Inc., New York.
2. Mollering, H. (1985) in Method of Enzymatic Analysis (Bergmeyer, H.K., ed..). 3rd ed., Vol II pp 39 - 47, Verlag Chemie, Weinheim, Deerfield Beach. Florida.
3. Olschimike, D., Niesner, W. and Junge, Ch. (1969) Bestimming der Appelsaure in wernen und Traubensaften, Deutsche Lebensmittel - Reindschaus **65**, 383-384.

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