EN

L-MALIC ACID

REF 984310 (for Gallery and Arena analyzers)

❶ 3 x 16 ml Reagent 1
❷ 3 x 4.5 ml Reagent 2
❸ 3 x 4.5 ml Reagent 3

REF 984311 (for Arena analyzers only)

❶ 3 x 45 ml Reagent 1
❷ 3 x 13 ml Reagent 2
❸ 3 x 13 ml Reagent 3

INTENDED USE

Reagent for photometric determination of L-Malic acid in homogenous liquid samples using automated Thermo Scientific™ Arena™ or Gallery™ analyzer.

METHOD

Enzymatic test with L-Malate-dehydrogenase (L-MDH) and Glutamate-Oxalacetate-Transaminase (GOT).
Method is performed at 37 °C, using 340 nm filter.

PRINCIPLE OF THE PROCEDURE

L-Malate + NAD+ <---L-MDH---> Oxalacetate + NADH + H+
Oxalactate + L-Glutamate <---GOT---> L-Aspartate + 2-Oxoglutarate

REAGENT INFORMATION

Reagent 1 (R1)	3 x 16 ml or 3 x 45 ml
Reagent 2 (R2)	3 x 4.5 ml or 3 x 13 ml
Reagent 3 (R3)	3 x 4.5 ml or 3 x 13 ml

Note: Labels of reagent vials have two barcodes.
For Arena analyzers, turn the short barcode to the barcode reader.
For Gallery analyzers, turn the long barcode to the barcode reader.

Concentrations

R1	Buffer	pH 10.3
	Glutamic acid	60 mmol/l
R2	NAD	≥ 20 mmol/l
R3	Buffer	pH 9.6
	GOT	≥ 10 KU/l
	L-MDH	≥ 150 KU/l

Precautions

The reagents contain sodium azide (< 0.1 %) as preservative. Do not swallow. Avoid contact with skin and mucous membranes. Take the necessary precautions for the use of laboratory reagents.

Preparation

The reagents R1, R2 and R3 are ready-to-use.
Note: Check that there are no bubbles on the surface of the reagent when you insert vials into the analyzer.

Storage and Stability

Reagents in unopened vials are stable at 2…8 °C until the expiry date printed on the label. Do not freeze the reagents.
Reagents are stable for 30 days on board.

SAMPLES

Sample Type

Food and other sample material.

Sample concentration and Arena/Gallery application

All method related details are in the separate application note.

Arena and Gallery applications have a primary dilution of 1+9, this means that every sample is automatically first diluted with 1+9.

Primary dilution and the Dilution limits Low and High can be changed according to the example table below if needed.

Dilution1+	Dilution limit (g/l)	
	Low	High
2	0.15	1.50
29	1.50	15.00

Sample preparation

If the sample has substances interfering the measurement, please handle it according to the following suitable preparation procedure:

- Use clear, colorless and practically neutral liquid samples directly.
- Filter or centrifuge turbid solutions.
- Degas samples containing carbon dioxide.
- Crush or homogenize solid or semi-solid samples.
- Weigh sufficient quantity of sample in a volumetric flask (take care of the measuring range), extract with water and filtrate, centrifuge..
- Weigh sufficient quantity of fat containing samples into a volumetric flask (take care of the measuring range), extract with hot water. Cool to allow the fat to separate, make up the mark, place the volumetric flask in an ice bath for 15 min. and filter.
- Adjust acid samples to pH 8 - 10 by adding sodium or potassium hydroxide solution and incubate for approx. 30 min.
- Treat strongly colored samples with polyvinylpolypyrrolidone (PVPP e.g. 1 g/100 ml Sample).
- **Because of the absorption of L-Malic acid, the Carrez clarification is not applicable.**

TEST PROCEDURE

See the separate Arena or Gallery System Application note for an automated procedure. Due to the differences in sample matrixes, all performance should be evaluated by the user.

Example of manul pipetting procedure (1 cm cuvette pathlenght, 37 °C, for sample concentrations 0.06 - 0.5 g/l):

	Reagent Blank (RB)	Sample	Sample Blank (SB, optional)
Sample / Standard	-	100 µl	100 µl
Dist. water	100 µl	-	-
Reagent 1	2000 µl	2000 µl	2000 µl
Reagent 2	500 µl	500 µl	500 µl
Mix and incubate for 1 min at 37 °C and read absorbance A1 at 340 nm. Continue by adding:			
Reagent 3	500 µl	500 µl	-
Dist. water	-	-	500 µl
Mix, and wait until the end of the reaction. Incubation time is approximately 5 min. Read the absorbance A2.			

For the manual, it is recommended to perform reagent blank in every run. It should be subtracted during calculation of the results. Sample blank is performed only when interferences by the sample itself are suspected.

Calculation for the manual method:

Measurement with RB: $\Delta A = (A_2 - df \times A_1)_{sample} - (A_2 - df \times A_1)_{RB}$

or with SB: $\Delta A = (A_2 - df \times A_1)_{sample} - (A_2 - df \times A_1)_{SB} - (A_2 - df \times A_1)_{RB}$

With df = dilution factor of the optical densities, because of reagent volumes. df = (sample volume + R1 + R2) / (sample volume + R1 + R2 +R3) = 0.839.

Calculation formula:

$$C_{L\text{-malic acid}}\,[g/l \text{ sample sol.}] = \frac{V \times MW \times \Delta A}{\varepsilon \times d \times v \times 1000}$$

with:
V (Total volume) = 3100 [µl]
MW (Molecular weight) = 134.09 [g/mol]
d (Optical path) = 1.00 [cm]
v (Sample volume) = 100 [µl]
ε (Extinction coefficient NADH) [l x $mmol^{-1}$ x cm^{-1}]: 340 nm = 6.3

Results for the determination at:
340 nm: $c_{L\text{-Malic acid}}$ [g/l] = 0.660 x ΔA

The above factors have to be recalculated again when changing parameters e.g. the sample volume. Please note that dilution factors of the sample preparations have to be considered in the calculation.

Materials required but not provided

Distilled water (aseptic and free of heavy metals) and general laboratory equipment.

Acid combination standard Cat no. 984382 (one level, water based) is not included in the kit.

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Calibration
Water based Acid combination standard can be used or other. Ordering code for Acid combination standard is 984382 (3x 3 ml). The standard is ready-to-use.

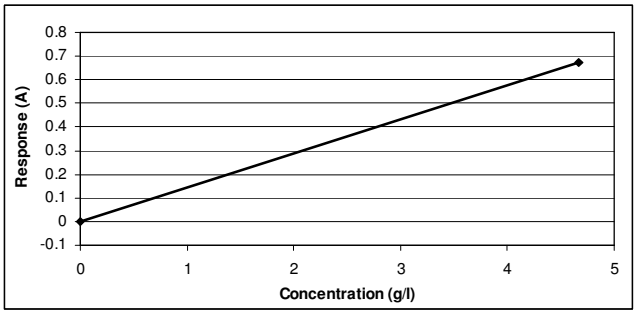
Quality Control
Use quality control samples at least once a day and after each calibration and every time a new bottle of reagent is used. It is recommended to use two level of controls. The control intervals and limits must be adapted to the individual laboratory requirements. The results of the quality control sample(s) should fall within the limits pre-set by the laboratory.

Available controls:
Acid combination standard can be used. If Acid combination standard is used also for calibration, an additional internal control is recommended to be used.

CALCULATION OF RESULTS
The results are calculated automatically by the analyzer using a calibration curve.

Conversion factors:
g/l x 7.4571 = mmol/l
mmol/l x 0.1341 = g/l

Calibration Curve (example)



Calibrator	Response (A)	Calc. conc. (g/l)
Water	-0.002	0.000
Acid std	0.672	4.670

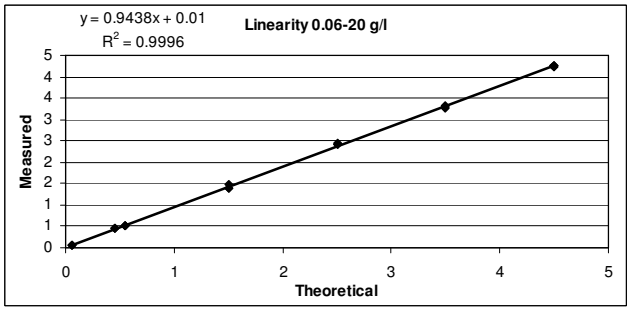
Calibration factor of this example is 0.935.
Note that the calibration curve is lot dependent.

LIMITATIONS OF THE PROCEDURE

Interference
The determination is specific for L-Malic acid. D-Malic acid does not react. No interferences were observed.

MEASURING RANGE
The test has been developed to determine L-Malic acid concentrations within a measuring range from 0.05 to 20 g/l.

PERFORMANCE CHARACTERISTICS
The results obtained in individual laboratories may differ from the performance data given.



Determination limit (=Test limit low)
The determination limit is the lowest concentration that can be measured quantitatively.
The determination limit for this method is 0.05 g/l.

Precision
Arena analyzer

	Mean 1.2 g/l		Mean 3.4 g/l	
	SD	CV %	SD	CV %
Within run	0.01	1.1	0.03	1.0

Gallery analyzer

	SD	CV %	SD	CV %	SD	CV %
Within run	0.011	0.9	0.0.23	0.9	0.024	0.6
Between run	0.017	1.4	0.035	1.4	0.065	1.7
Total	0.020	1.7	0.042	1.7	0.069	1.8

A precision study was performed using Gallery for 5 days, with the number of measurements being n = 50.

OTHER REMARKS
Note that the application performance has been verified with pure chemicals dissolved in deionized water and spiked native samples. The results obtained in individual laboratories may differ from the given performance data due to e.g. sample matrix, concentrations or analysis environment. Each laboratory is responsible to verify the method to prove the analysis performance.

WASTE MANAGEMENT
Please refer to local legal requirements. It is recommended to empty the analyzer cuvette waste bin and waste water daily. Emptying should be done immediately after the analysis when using hazardous reagents/solutions.
Note: If using reagents/solutions that react with each other, cuvette waste bin and waste water should be emptied and washed between use of these reagents.
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ADDITIONAL MATERIAL
Certificate of analysis and SDS are available at
www.e-labeling.eu/TSF

Applications for Gallery and Arena automated analyzers are available upon request from the local sales representative. Information in the Application note can change without prior notice.

MANUFACTURER
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Date of revision (yyyy-mm-dd)
2014-03-21

Changes from previous version
Sample preparation section updated.
General updates.

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