SNAPSHOOT
ENGINEERED FOR A SAFER TOMORROW

현장을 이해하고, 필요한 솔루션을 연결합니다.

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COMPANY INFORMATION

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제품의 규격과 성능, 인증 및 적용 조건을 확인하기 위한 자료입니다.

ABOUT SNAPSHOOT

제품 공급을 넘어, 현장에 맞는 해결책으로

스냅슛은 산업현장의 작업 환경과 요구 조건을 바탕으로
적합한 제품과 시스템을 제안합니다. 현장 실사를 통해 설치 조건을 확인하고,
공급부터 시공·설치 및 사용 교육까지 필요한 과정을 함께합니다.

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Megazyme

www.megazyme.com

L-MALIC ACID (L-MALATE)

ASSAY PROCEDURE

K-LMAL-58A/K-LMAL-116A 09/19

(*58/116 Manual Assays per Kit) or
(580/1160 Microplate Assays per Kit)

** The number of tests per kit can be doubled if all volumes are halved*



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INTRODUCTION:

As a component of the citric acid cycle, L-malic acid (L-malate) is found in all living organisms. Its quantitative determination is especially important in the manufacture of wine, beer, bread, fruit and vegetable products, as well as in cosmetics and pharmaceuticals. It is one of the most important fruit acids and has the highest concentration of all acids in wine. In the wine industry, the level of L-malic acid is monitored, along with L-lactic acid, during malolactic fermentation. L-Malic acid finds many applications as a food preservative (E296) and flavour enhancing compound, such as in the manufacture of low calorie drinks.

This booklet describes a manual spectrophotometric and a microplate method for the quantitative determination of L-malic acid. However, an alternative method tailored towards the small winery is also available from Megazyme that is based on the use of an inexpensive colorimeter, the MegaQuant™ meter (see product **D-LMALMQ**). A third kit for L-malic acid has been optimised for auto-analyser applications (see product **K-LMALAF**). For further information regarding these alternative L-malic acid procedures, see the relevant booklet at ***www.megazyme.com***.

PRINCIPLE:

The detection of L-malic acid requires two enzyme reactions. In the first reaction catalysed by L-malate dehydrogenase (L-MDH), L-malic acid is oxidised to oxaloacetate by nicotinamide-adenine dinucleotide (NAD^+) (1).

$$\text{(1)}\quad \text{L-Malic acid} + NAD^+ \xleftrightarrow{\text{(L-MDH)}} \text{oxaloacetate} + NADH + H^+$$

However, since the equilibrium of reaction (1) lies firmly in the favour of L-malic acid and NAD^+, a further reaction is required to "trap" the NADH product, and this is achieved by the conversion of oxaloacetate to L-aspartate and 2-oxoglutarate, in the presence of a large excess of L-glutamate, by glutamate-oxaloacetate transaminase (GOT) (2).

$$\text{(2)}\quad \text{Oxaloacetate} + \text{L-glutamate} \xrightarrow{\text{(GOT)}} \text{L-aspartate} + \text{2-oxoglutarate}$$

The amount of NADH formed in the above coupled reaction is stoichiometric with the amount of L-malic acid. It is the NADH which is measured by the increase in absorbance at 340 nm.

Methods based on this principle are recommended by IFU, AIJN, MEBAK and OIV, and approved by AOAC International. The method is contained in the food laws of many countries and in European regulations.

SPECIFICITY, SENSITIVITY, LINEARITY AND PRECISION:

The assay is specific for L-malic acid. D-Malic acid, L-lactic acid, L-aspartic acid and fumaric acid do not react.

The smallest differentiating absorbance for the assay is 0.005 absorbance units. This corresponds to 0.12 mg/L of sample solution at the maximum sample volume of 2.00 mL (or to 2.49 mg/L with a sample volume of 0.10 mL). The detection limit is 0.25 mg/L, which is derived from an absorbance difference of 0.010 and the maximum sample volume of 2.00 mL.

The assay is linear over the range of 0.5 to 30 µg of L-malic acid per assay. In duplicate determinations using one sample solution, an absorbance difference of 0.005 to 0.010 may occur. With a sample volume of 0.10 mL, this corresponds to a L-malic acid concentration of approx. 2.49 to 4.98 mg/L of sample solution. If the sample is diluted during sample preparation, the result is multiplied by the dilution factor, F. If, in sample preparation, the sample is weighed, e.g. 10 g/L, a difference of 0.02 to 0.05 g/100 g can be expected.

INTERFERENCE:

If the conversion of L-malic acid has been completed within the time specified in the assay (approx. 3 min), it can be generally concluded that no interference has occurred. However, this can be further checked by adding L-malic acid (approx. 15 µg in 0.10 mL) to the cuvette on completion of the reaction. A significant increase in the absorbance should be observed.

Interfering substances in the sample being analysed can be identified by including an internal standard. Quantitative recovery of this standard would be expected. Losses in sample handling and extraction are identified by performing recovery experiments, i.e. by adding L-malic acid to the sample in the initial extraction steps. Polyvinylpyrrolidone has been incorporated into the assay system to prevent inhibition from polyphenolics (tannins) in the sample.

SAFETY:

The general safety measures that apply to all chemical substances should be adhered to.

For more information regarding the safe usage and handling of this product please refer to the associated SDS that is available from the Megazyme website.

KITS:

Kits suitable for performing 58/116 assays in manual format (or 580/1160 assays in microplate format) are available from Megazyme. The kits contain the full assay method plus:

58 Determinations Kit (cat. no. K-LMAL-58A)

Bottle 1: Buffer (6 mL, pH 10.0) plus L-glutamate and sodium azide (0.02% w/v) as a preservative.
Stable for > 2 years at 4°C.

Bottle 2: NAD^+ plus PVP.
Stable for > 5 years below -10°C.

Bottle 3: Glutamate-oxaloacetate transaminase suspension (1.25 mL).
Stable for > 2 years at 4°C.

Bottle 4: L-Malate dehydrogenase suspension (1.25 mL).
Stable for > 2 years at 4°C.

Bottle 5: L-Malic acid standard solution (5 mL, 0.15 mg/mL).
Stable for > 2 years; store sealed at 4°C.

116 Determinations Kit (cat. no. K-LMAL-116A)

Bottle 1: Buffer (12 mL, pH 10.0) plus L-glutamate and sodium azide (0.02% w/v) as a preservative.
Stable for > 2 years at 4°C.

Bottle 2: (x2) NAD^+ plus PVP.
Stable for > 5 years below -10°C.

Bottle 3: Glutamate-oxaloacetate transaminase suspension (2.5 mL).
Stable for > 2 years at 4°C.

Bottle 4: L-Malate dehydrogenase suspension (2.5 mL).
Stable for > 2 years at 4°C.

Bottle 5: L-Malic acid standard solution (5 mL, 0.15 mg/mL).
Stable for > 2 years; store sealed at 4°C.

PREPARATION OF REAGENT SOLUTIONS/SUSPENSIONS:

1. Use the contents of bottle 1 as supplied.
Stable for > 2 years at 4°C.

2. Dissolve the contents of bottle 2 in 6 mL of distilled water. **Stable for > 1 year at 4°C** or stable for > 2 years below -10°C (to avoid repetitive freeze/thaw cycles, divide into appropriately sized aliquots and store in polypropylene tubes). **Do not** dissolve the contents of the second bottle (116 determinations kit only) until required.

3 & 4. Use the contents of bottles 3 and 4 as supplied. Before opening for the first time, shake the bottles to remove any enzyme that may have settled on the rubber stopper. Subsequently, store the bottles in an upright position. Stable for > 2 years sealed at 4°C.

5. Use the contents of bottle 5 as supplied. Stable for > 2 years; store sealed at 4°C.

NOTE: The L-malic acid standard solution is only assayed where there is some doubt about the accuracy of the spectrophotometer being used or where it is suspected that inhibition is being caused by substances in the sample. The concentration of L-malic acid is determined directly from the extinction coefficient of NADH (see page 6).

EQUIPMENT (RECOMMENDED):

1. Glass test tubes (round bottomed; 16 x 100 mm).
2. Disposable plastic cuvettes (1 cm light path, 3.0 mL).
3. Micro-pipettors, e.g. Gilson Pipetman® (20 µL and 100 µL).
4. Positive displacement pipettor, e.g. Eppendorf Multipette®
   - with 5.0 mL Combitip® (to dispense 0.1 mL aliquots of buffer 1 and NAD^+ solution).
   - with 25 mL Combitip® (to dispense 2.0 mL aliquots of distilled water).
5. Analytical balance.
6. Spectrophotometer set at 340 nm.
7. Vortex mixer (e.g. IKA® Yellowline Test Tube Shaker TTS2).
8. Stop clock.
9. Whatman No. 1 (9 cm) filter papers.

A. MANUAL ASSAY PROCEDURE:

Wavelength: 340 nm
Cuvette: 1 cm light path (glass or plastic)
Temperature: ~ 25°C
Final volume: 2.34 mL
Sample solution: 0.5-30 µg of L-malic acid per cuvette (in 0.10-2.0 mL sample volume)

Read against air (without a cuvette in the light path) or against water

Pipette into cuvettes	Blank	Sample
distilled water (at ~ 25°C)	2.10 mL	2.00 mL
sample	-	0.10 mL
solution 1 (buffer)	0.10 mL	0.10 mL
solution 2 (NAD^+/PVP)	0.10 mL	0.10 mL
suspension 3 (GOT)	0.02 mL	0.02 mL
Mix*, read the absorbances of the solutions (A_1) after approx. 3 min and start the reactions by addition of:		
suspension 4 (L-MDH)	0.02 mL	0.02 mL
Mix* and read the absorbances of the solutions (A_2) at the end of the reaction (approx. 3 min).		

* for example with a plastic spatula or by gentle inversion after sealing the cuvette with a cuvette cap or Parafilm®.

CALCULATION:

Determine the absorbance difference (A_2-A_1) for both blank and sample. Subtract the absorbance difference of the blank from the absorbance difference of the sample, thereby obtaining $\Delta A_{\text{L-malic acid}}$. The value of $\Delta A_{\text{L-malic acid}}$ should as a rule be at least 0.100 absorbance units to achieve sufficiently accurate results.

The concentration of L-malic acid can be calculated as follows:

$$c = \frac{V \times MW}{\varepsilon \times d \times v} \times \Delta A_{\text{L-malic acid}} \quad [g/L]$$

where:

V = final volume [mL]
MW = molecular weight of L-malic acid [g/mol]
ε = extinction coefficient of NADH at 340 nm
= 6300 [l x mol^{-1} x cm^{-1}]
d = light path [cm]
v = sample volume [mL]

It follows for L-malic acid:

$$c = \frac{2.34 \times 134.09}{6300 \times 1.0 \times 0.1} \times \Delta A_{\text{L-malic acid}} \quad [g/L]$$

$$= 0.4980 \times \Delta A_{\text{L-malic acid}} \quad [g/L]$$

If the sample has been diluted during preparation, the result must be multiplied by the dilution factor, F.

When analysing solid and semi-solid samples which are weighed out for sample preparation, the content (g/100 g) is calculated from the amount weighed as follows:

Content of L-malic acid

$$= \frac{c_{\text{L-malic acid}}\ [g/L\ \text{sample solution}]}{\text{weight}_{\text{sample}}\ [g/L\ \text{sample solution}]} \times 100 \quad [g/100\ g]$$

NOTE: These calculations can be simplified by using the Megazyme ***Mega-Calc***™, downloadable from where the product appears on the Megazyme website (www.megazyme.com).

B. MICROPLATE ASSAY PROCEDURE:

NOTES:

1. The Microplate Assay Procedure for L-malic acid can be performed using either a single point standard or a full calibration curve.
2. For each batch of samples that is applied to the determination of L-malic acid **either a single point standard or a calibration curve must be performed concurrently using the same batch of reagents.**

Wavelength: 340 nm
Microplate: 96-well (e.g. clear flat-bottomed, glass or plastic)
Temperature: ~ 25°C
Final volume: 0.234 mL
Linearity: 0.1-3 µg of L-malic acid per well (in 0.01-0.20 mL sample volume)

Pipette into wells	Blank	Sample	Standard
distilled water	0.210 mL	0.200 mL	0.200 mL
sample solution	-	0.010 mL	-
standard solution	-	-	0.010 mL
solution 1 (buffer)	0.010 mL	0.010 mL	0.010 mL
solution 2 (NAD^+/PVP)	0.010 mL	0.010 mL	0.010 mL
suspension 3 (GOT)	0.002 mL	0.002 mL	0.002 mL
Mix*, read the absorbances of the solutions (A_1) after approx. 3 min and start the reactions by addition of:			
solution 4 (L-MDH)	0.002 mL	0.002 mL	0.002 mL
Mix* and read the absorbances of the solutions (A_2) at the end of the reaction (approx. 3 min). If the reaction has not stopped after 3 min, continue to read the absorbances at 1 min intervals until the absorbances increase constantly over 1 min**.			

* for example using microplate shaker, shake function on a microplate reader, or repeated aspiration (e.g. using a pipettor set at 50-100 µL volume).

** if this "creep" rate is greater for the sample than for the blank, extrapolate the sample absorbances back to the time of addition of solution 4.

CALCULATION (Microplate Assay Procedure):

$$g/L = \frac{\Delta A_{sample}}{\Delta A_{standard}} \times g/L \text{ standard} \times F$$

If the sample is diluted during preparation, the result must be multiplied by the dilution factor, F.

SAMPLE PREPARATION:

1. Sample dilution.

The amount of L-malic acid present in the cuvette (i.e. in the 0.1 mL of sample being analysed) should range between 0.5 and 30 µg. The sample solution must therefore be diluted sufficiently to yield an L-malic acid concentration between 0.005 and 0.30 g/L.

Dilution Table

Estimated concentration of L-malic acid (g/L)	Dilution with water	Dilution factor (F)
< 0.3	No dilution required	1
0.3-3.0	1 + 9	10
3.0-30	1 + 99	100
> 30	1 + 999	1000

If the value of $\Delta A_{\text{L-malic acid}}$ is too low (e.g. < 0.100), weigh out more sample or dilute less strongly. Alternatively, the sample volume to be pipetted into the cuvette can be increased up to 2.00 mL, making sure that the sum of the sample and distilled water components in the reaction is 2.10 mL and using the new sample volume in the equation.

2. Sample clarification.

a. Solutions:

Carrez I solution. Dissolve 3.60 g of potassium hexacyanoferrate (II) $\{K_4[Fe(CN)_6].3H_2O\}$ (Sigma cat. no. P9387) in 100 mL of distilled water. Store at room temperature.

Carrez II solution. Dissolve 7.20 g of zinc sulphate ($ZnSO_4.7H_2O$) (Sigma cat. no. Z4750) in 100 mL of distilled water. Store at room temperature.

Sodium hydroxide (NaOH, 100 mM). Dissolve 4 g of NaOH in 1 L of distilled water. Store at room temperature.

b. Procedure:

Pipette the liquid sample into a 100 mL volumetric flask which contains approx. 60 mL of distilled water or weigh sufficient quantity of the sample into a 100 mL volumetric flask and add 60 mL of distilled water. Carefully add 5 mL of Carrez I solution, 5 mL of Carrez II solution and 10 mL of NaOH solution (100 mM). Mix after each addition. Fill the volumetric flask to the mark, mix and filter.

3. General considerations.

(a) Liquid samples: clear, slightly coloured and approximately neutral, liquid samples can be used directly in the assay.

(b) Acidic samples: if > 0.1 mL of an acidic sample is to be used undiluted (such as wine or fruit juice), the pH of the solution should be increased to approx. 9.0 using 2 M NaOH, and the solution incubated at room temperature for 30 min.

(c) Carbon dioxide: samples containing significant quantities of carbon dioxide, such as beer, should be degassed by increasing the pH to approx. 9.0 with 2 M NaOH and gentle stirring, or by stirring with a glass rod.

(d) Coloured samples: an additional sample blank, i.e. sample with no L-MDH, may be necessary in the case of coloured samples.

(e) Strongly coloured samples: if used undiluted, strongly coloured samples should be treated by the addition of 0.2 g of PVPP/10 mL of sample. Shake the tube vigorously for 5 min and then filter through Whatman No. 1 filter paper.

(f) Solid samples: homogenise or crush solid samples in distilled water and filter if necessary.

(g) Samples containing fat: extract such samples with hot water at a temperature above the melting point of the fat, e.g. in a 100 mL volumetric flask. Adjust to room temperature and fill the volumetric flask to the mark with distilled water. Store on ice or in a refrigerator for 15-30 min and then filter. Discard the first few mL of filtrate and use the clear supernatant (which may be slightly opalescent) for assay. Alternatively, clarify with Carrez reagents.

(h) Samples containing protein: deproteinise samples containing protein by adding an equal volume of ice-cold 1 M perchloric acid with mixing. Centrifuge at 1,500 *g* for 10 min and neutralise the supernatant with 1 M KOH. Alternatively, use Carrez reagents.

SAMPLE PREPARATION EXAMPLES:

(a) Determination of L-malic acid in wine.

The free L-malic acid concentration [F] of white and red wine can generally be determined without any sample treatment (except dilution according to the dilution table). *Typically, a dilution of 1:10 and sample volume of 0.1 mL are satisfactory.*

(b) Determination of L-malic acid and its esterified derivatives in wine.

The concentration of both free and esterified L-malic acid [F + E] in white and red wine can be determined as follows: add 6 mL of 2 M NaOH to 20 mL of wine and heat under reflux for 30 min with stirring. After cooling, carefully adjust the pH of the solution to 10 with 1 M H_2SO_4 and adjust the volume to 50 mL with distilled water.

Then analyse the sample according to the general procedure. The concentration obtained is the sum of the free and esterified L-malic acid [F + E], and thus the esterified L-malic acid concentration alone [E] can be calculated as follows:

$$[E] = [F + E] - [F] \qquad [g/L]$$

(c) Determination of L-malic acid in fruit juice, concentrates and related beverages.

The L-malic acid concentration of clear, neutral solutions can generally be determined without any sample treatment (except dilution according to the dilution table). Turbid liquids generally only require filtering before the dilution step. Coloured solutions are usually suitable for analysis after dilution to an appropriate L-malic acid concentration. However, if such solutions require analysis undiluted, they may need decolourising as follows: add 0.2 g of PVPP/10 mL of sample; shake the tube vigorously for 5 min and then filter through Whatman No. 1 filter paper. Use the clear/slightly coloured filtrate directly in the assay. *Typically, a dilution of 1:50 and sample volume of 0.1 mL are satisfactory.*

(d) Determination of L-malic acid in beer.

Remove carbon dioxide by stirring with a glass rod and dilute if necessary. *Typically, no dilution is required and a sample volume of 0.1 mL is satisfactory.*

(e) Determination of L-malic acid in solid foodstuffs.

Homogenise approx. 10 g of solid foodstuff using a mortar and pestle or an electric blender. Extract approx. 2 g of representative material (weighed accurately) in 40 mL of distilled water for 30 min, with heating at 60°C where necessary. Quantitatively transfer the extract to a 50 mL volumetric flask and adjust to volume with distilled water. Filter the turbid solution and dilute if necessary.

REFERENCES:

1. Mollering, H. (1985). L-Malate. "*Methods of Enzymatic Analysis"* (Bergmeyer, H. U., ed.), 3rd ed., **Vol. VII**, pp. 39-47, VCH Publishers (UK) Ltd., Cambridge, UK.
2. AOAC Official Methods of Analysis (2002). Method 993.05 "*L-Malic acid/total malic acid ratio in apple juice*", 17th ed., Chapter 37, p. 15.
3. Gorin, N. (1976). Differences in L-malate determined enzymatically or titrimetrically in golden delicious apples. *Zeitschrift fur Lebensmittel-Unterschung und-Forschung,* **162**, 259-261.

4. Klopper, W. J., Angelino, S. A. G. F., Tuning, B. & Vermeire, H. A. (1986). Organic acids and glycerol in beer. *J. Inst. Brew.* **92**, 225-228.

5. Elkins, E. R. & Freund, W. (1994). Detection of adulteration in apple juice by L-malic/total malic acid ratio: Collaborative Study. *J. AOAC Int.* **77**, 411-415.

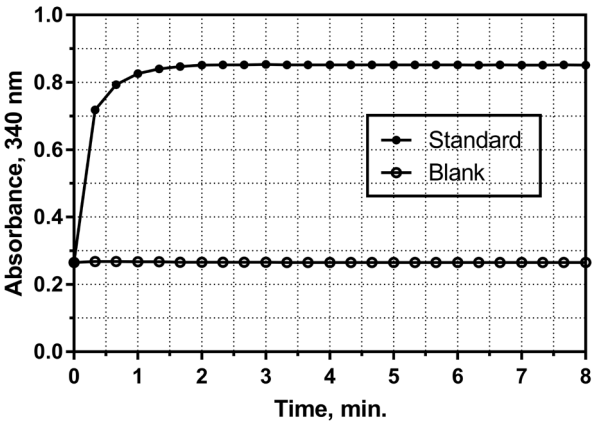


Figure 1. Increase in absorbance at 340 nm on incubation of 30 µg of L-malic acid with L-malate dehydrogenase in the presence of NAD^+.

NOTE:

The assay procedure described above requires the use of a UV/Visible spectrophotometer to allow measurement of absorbance at 340 nm. Since such equipment is not likely to be available in smaller wineries, we have modified the assay procedure to include an additional reaction in which diaphorase is employed to catalyse the formation of an INT-formazan (a red-coloured compound) from NADH and INT (iodonitrotetrazolium chloride). This reaction is quantitative, and the production of a red-coloured compound allows the use of a simple colourimeter. This product (**K-LMALMQ**) and a simple colourimeter with reagents (**D-LMALMQ**) is offered by Megazyme as ' L-Malic Acid - MegaQuant™ Format'.

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