Megazyme

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MegaQuant™

L-MALIC ACID (L-MALATE)

ASSAY PROCEDURE

K-LMALMQ 08/18

(60 Assays per Kit)

Megazyme

INTRODUCTION:

In the wine industry, the level of L-malic acid is monitored, along with L-lactic acid, during malolactic fermentation. The assay procedure described here is specifically aimed at this industry for the measurement of L-malic acid in the latter stages of malolactic fermentation. However, the procedure is also applicable to measurement of L-malic acid in a diverse range of food and beverage matrices. The assay is simple to perform, rugged, reliable, accurate, and employs an inexpensive colorimeter.

PRINCIPLE:

The detection of L-malic acid using the MegaQuant™ assay format requires three 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).

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

In the third reaction, in the presence of diaphorase, NADH reduces iodonitrotetrazolium chloride (INT) to an INT-formazan compound which absorbs in the range of 480-520 nm (3).

$$\text{(3)}\quad NADH + INT + H^+ \xrightarrow{\text{(diaphorase)}} NAD^+ + \text{INT-formazan}$$

The amount of INT-formazan formed in this reaction is stoichiometric with the amount of L-malic acid. It is the INT-formazan which is measured by the increase in absorbance at 505 nm.

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 0.01 absorbance units. This corresponds to 7.7 mg/L of sample solution with a sample

volume of 20 µL. The detection limit is 15.4 mg/L, which is derived from an absorbance difference of 0.02 and a sample volume of 20 µL.

The assay is linear over the range of 0.15 to 15 µg of L-malic acid per assay (i.e. 0.007-0.75 g/L with a 20 µL sample volume). In duplicate determinations using one sample solution, an absorbance difference of 0.01 to 0.02 may occur. With a sample volume of 20 µL, this corresponds to a L-malic acid concentration of approx. 7.7-15.4 mg/L of sample solution. If the sample is diluted during sample preparation, the result is multiplied by the dilution factor, F.

INTERFERENCE:

Phenolics, especially in red wine, interfere with the assay as they react with INT causing a "creep" reaction (Figure 2, page 10). For red wines, this reaction is so significant that if undiluted wine is to be analysed, phenolics must first be removed with polyvinylpolypyrrolidone (PVPP) [see sample preparation, example (b), page 11, red wine]. A "creep" reaction is also seen when analysing some undiluted white wines. However, this is so slow that it is accounted for in the recommended assay format and the associated calculations.

In the measurement of L-malic acid using the MegaQuant™ procedure, it is essential to recognise and account for two potential sources of minor error, namely:

1. While PVPP removes most of the phenolics from red wine, sufficient remains to cause a slight "creep" reaction. Since this reaction is very slow in PVPP treated wines (leading to a potential overestimation of L-malic acid of only 0.00 to 0.01 g/L for white wine, or 0.0 to 0.03 g/L for red wine), it can be ignored (see Assay Format A). However, if desired, this can be accurately accounted for by using assay format B.

2. Using the PVPP tablets as recommended in these assay formats (i.e. 1 tablet per 5 mL of wine), results in a 2% (v/v) dilution of solution concentration. This is allowed for in the calculations (see PVPP treated samples; pages 7 and 9).

Reducing substances such as sulphite and ascorbate can interfere with the assay, but only at levels not experienced in wine, e.g. sulphite at a concentration of 4 g/L gives a slight creep reaction [which can be accounted for (see Assay Format B, page 8)]. Samples containing very high levels of ascorbate (> 400 mg/L) cannot be assayed with this procedure.

The significance of other interferences can be checked by adding L-malic acid (7.5 µg in 20 µL) to the cuvette on completion of the reaction. A substantial 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 should be achieved.

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 60 determinations are available from Megazyme. The kits contain the full assay method plus:

Bottle 1: Buffer (100 mL, pH 10.0) plus L-glutamate and sodium azide (0.02% w/v) as a preservative.
Stable for > 2 years at 4°C.

NOTE: This buffer contains sodium azide (0.02% w/v) as a preservative, and thus should **not** be dispensed using a mouth pipette.

Bottle 2: Test tablets (60) containing diaphorase, GOT, INT, FAD and NAD^+. These are supplied in a special plastic vial containing impregnated desiccant. Allow this container to warm to room temperature (preferably in the presence of a desiccant) before opening to remove tablets. This will ensure that remaining tablets will not absorb moisture and thus guarantee maximum stability.
Stable for > 2 years when stored dry in a domestic freezer (below -10°C).

Bottle 3: L-Malate dehydrogenase suspension (1.3 mL).
Stable for > 2 years at 4°C.

Bottle 4: L-Malic acid standard solution (5 mL, 0.375 mg/mL).
Stable for > 2 years at 4°C.

Bottle 5: Tablets (65) containing PVPP.
Stable for > 5 years at room temperature.

PREPARATION OF REAGENT SOLUTIONS/SUSPENSIONS:

1. Use the contents of bottle 1 as supplied (1.5 mL per assay); or **alternatively**, dilute this solution with an equal volume of purified water and then use 3.0 mL per assay. Stable for > 2 years at 4°C.

2. Use the contents of bottle 2 as supplied. Stable for > 2 years when stored dry in a domestic freezer.

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

4. Use the contents of bottle 4 as supplied (20 µL per test). Warm to room temperature before dispensing. Stable for > 2 years at 4°C.

5. Use the contents of bottle 5 as supplied. Stable for > 5 years at room temperature.

NOTE: The L-malic acid standard solution is only assayed where there is some doubt about the accuracy of the colorimeter or where it is suspected that inhibition is being caused by substances in the sample. The concentration of L-malic acid is determined using the equation on page 7 (derived from the standard curve on page 12).

EXTRA EQUIPMENT/REAGENTS REQUIRED:

1. Special glass colorimeter test tubes (flat bottomed; 16 x 100 mm) as supplied with the instrument. Extra tubes are available from Megazyme (cat. no. **D-MQTUB**).
2. Disposable, graduated 10 mL polypropylene tubes, e.g. Sarstedt cat. no. 62.551.201 PP (www.sarstedt.com).
3. Micro-pipettor, e.g. Gilson Pipetman® (20 µL), to dispense 20 µL of sample, standard and L-malate dehydrogenase.
4. Eppendorf Multipette® positive displacement pipettor
   - with 25 mL Combitip® (to dispense 1.5 mL aliquots of solution 1 and 1.5 mL aliquots of purified water).
5. Glass graduated pipette (5 mL) and Pipette Pump II (10 mL, green; Sigma cat. no. P7924); or Pipette filler (10 mL, green; Merck Eurolab/VWR International, cat. no. 241/3983/03) for use with glass pipettes.
6. MegaQuant™ colorimeter (reading at 505 nm).
7. Stop clock or watch.

NOTES:

1. The format described here is specifically designed for analysing wines approaching the end of malolactic fermentation. Analysis of wine containing > 0.75 g of L-malic acid/L requires suitable dilution of the sample in purified water (distilled water or good quality, commercially available, filtered drinking water).
2. Purified water (distilled water or good quality, commercially available, filtered drinking water) will be required in the assay and for dilution of grape juice or wine samples. If using drinking water, first check the suitability of this by running a test with the D-fructose standard.
3. Colorimeter tubes must be clean and dry before use. The tubes should be polished clean with tissue paper.

PROCEDURE:

REMOVAL OF PHENOLICS:
(generally required only for red wines)

1. Transfer approx. 5 mL of red wine to a graduated 10 mL polypropylene tube (equipment 2), using the graduations on the tube as a guide.
2. Add one PVPP tablet (bottle 5), cap the tube and mix the contents by continuous inversion over **5 min**. Filter the contents of the tube through a Whatman No. 1 (9 cm) filter circle. Collect approx. 1 mL of filtrate. The filtrate must be no darker in colour than a light pink (which is generally the case). However, intensely coloured wines may require the addition of two PVPP tablets per 5 mL of wine. In such cases, to obtain sufficient filtrate (approx. 1 mL), it is necessary to carefully squeeze the folded filter circle with a spatula.
3. Analyse 20 µL of the filtrate.

A. Assay Format A: (*simple format - see Figure 1, page 8*)

1. Transfer **1.5 mL** of Solution 1 (at ~ 23°C or higher) and 1.5 mL of purified water [using an Eppendorf Multipette® with 25 mL Combitip® (equipment 4) or a 5 mL graduated glass pipette with pipette filler (equipment 5)] to a colorimeter test tube.

   Alternatively, transfer **3.0 mL** of Solution 1 that has been diluted with an equal volume of purified water (see Preparation of Reagent Solutions/Suspensions, point 1, page 4) to the test tube.
2. Using the supplied forceps, add a reagent test tablet (bottle 2) and allow this to dissolve over approx. 1-2 min. Swirl the tube intermittently to ensure **complete** dissolution.

3. Add 20 µL of wine sample [PVPP treated (undiluted red or white wine), non-PVPP treated (undiluted white wine sample) or diluted wine or grape juice sample] and swirl the tube to ensure thorough mixing.
4. After approx. 1 min, insert the tube into the MegaQuant™ colorimeter and press the **ON** key until the '---' symbol appears on the display (2 sec).
5. Release the ON key when the display shows '0.00' (this is $\mathbf{A_1}$).
6. Remove the tube from the colorimeter and add 20 µL of the contents of bottle 3 (L-malate dehydrogenase). Swirl the tube to ensure complete mixing. At the same time, press the start button on a stop clock timer, or record the time on a wrist watch.
7. Push the tube back into the colorimeter, and after 5 min, press the **ON** key until the display appears (1 sec). Record the reading in a records book as $\mathbf{A_2}$.
8. To ensure that the reaction has come to completion, take extra readings at 1 min intervals beyond the 5 min reading. The reading should remain the same over a 1 min interval (Fig. 1, page 8 and Fig. 2, page 10).

NOTE:

1. If the value of $\mathbf{A_2}$ is > 1.00, dilute the sample 10-fold more and repeat the test. If the value of $\mathbf{A_2}$ is < 0.10, use a less diluted sample (e.g. 10-fold diluted instead of 100-fold).
2. The colour of the assay solution **will** increase over longer periods of time due to the light sensitivity of INT (the chromogen in the tablet). It is thus necessary to take readings within the time frame recommended in the method.
3. Before running the test, bottle 1 (page 3) should be removed from the refrigerator and allowed to warm to room temperature. If the temperature is < 23°C, the reaction proceeds less rapidly.
4. Perform a **reagent blank** by adding a test tablet to 1.5 mL of solution 1 plus 1.52 mL of purified water. Zero the colorimeter after the tablet has completely dissolved. Then add 20 µL of L-malate dehydrogenase (bottle 3) and measure the absorbance increase (A_2) after 5 min. The absorbance change (A_2) should be insignificant (i.e 0.00 to 0.01).

CALCULATION:

$\Delta A_{\text{L-malic acid}}$ = the absorbance increase (A_2)

The value of $\Delta A_{\text{L-malic acid}}$ should as a rule be at least 0.10 absorbance units to achieve sufficiently accurate results.

The concentration of L-malic acid in the sample being analysed is determined using the equations below (derived from the standard curve in Figure 3; page 12):

*Concentration of L-malic acid for **non-PVPP** treated samples.*

with 20 µL sample volume and no sample dilution:

$$c = 0.771 \times \Delta A_{\text{L-malic acid}} \quad [g/L]$$

with 20 µL sample volume and with sample dilution:

$$c = 0.771 \times \text{dilution factor} \times \Delta A_{\text{L-malic acid}} \quad [g/L]$$

*Concentration of L-malic acid for **PVPP** treated samples.*

with 20 µL sample volume and no sample dilution:

$$c = 0.771 \times \Delta A_{\text{L-malic acid}} \times 102/100 \quad [g/L]$$
$$= 0.786 \times \Delta A_{\text{L-malic acid}} \quad [g/L]$$

with 20 µL sample volume and with sample dilution:

$$c = 0.786 \times \text{dilution factor} \times \Delta A_{\text{L-malic acid}} \quad [g/L]$$

where:

102/100 = allowance for the dilution of wine by PVPP treatment.

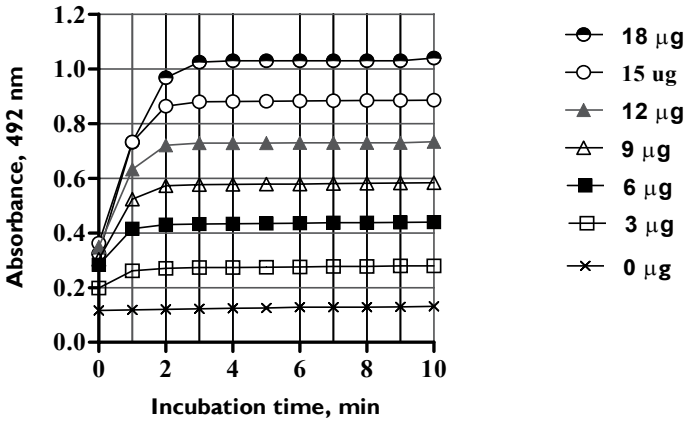

Figure 1. Time course of colour formation in the MegaQuant™ L-malic acid test performed in a recording spectrophotometer (25°C, 1 cm light path). **NOTE:** for a given amount of L-malic acid, the absorbance values are lower in this Figure than in Figure 3. This is because in this assay the cuvette path-length is 1 cm, whereas in the MegaQuant™ colorimeter test tubes it is approx. 1.3 cm.

ASSAY FORMAT B: (*"creep" corrected*)

1. Transfer 1.5 mL of Solution 1 and 1.5 mL of purified water (see point 1, top of page 4) [using an Eppendorf Multipette® with 25 mL Combitip® or a 5 mL graduated glass pipette with pipette filler (Equipment 5)] to a colorimeter test tube.

   Alternatively, transfer **3.0 mL** of Solution 1 that has been diluted with an equal volume of purified water (see Preparation of Reagent Solutions/Suspensions, point 1, page 4) to the test tube.

2. Using the supplied forceps, add a reagent test tablet (bottle 2) and allow this to dissolve over approx. 1-2 min. Swirl the tube intermittently to ensure **complete** dissolution.

3. Add **20 µL** of PVPP treated red or white wine sample and swirl the tube to ensure thorough mixing.

4. After approx. 1 min, insert the tube into the MegaQuant™ colorimeter and press the **ON** key until the '---' symbol appears on the display (2 sec).

5. Release the **ON** key when the display shows '0.00' (this is $\mathbf{A_0}$). At the same time, press the start button on a stop clock timer, or record the time on a wrist watch.

6. Leave the tube in the colorimeter for exactly 5 min and then press the **ON** key until the display appears (1 sec). Do not hold the key down or the instrument will blank on the sample and cause errors in readings. Record the reading in a records book as $\mathbf{A_1}$ (this is the increase in colour due to the creep reaction over 5 min).

7. Remove the tube from the colorimeter and immediately add 20 µL of L-malate dehydrogenase (bottle 3). Swirl the tube to ensure complete mixing. At the same time, press the start button on a stop clock timer, or record the time on a wrist watch.

8. Push the tube back into the colorimeter, and after 5 min, press the **ON** key to display the reading (absorbance) for the sample, $\mathbf{A_2}$. Record this value.

9. To confirm that the reaction has come to completion (Fig. 1, page 8 and Fig. 2, page 10), take extra readings at 1 min intervals beyond the 5 min reading. This reading should increase by no more than 0.01 absorbance units over a 1 min interval.

CALCULATION:

Determine $\Delta A_{\text{L-malic acid}}$ as the absorbance difference: $[(A_2\text{-}A_1) - (A_1\text{-}A_0)]$. Since the meter is zeroed on A_0, the equation becomes $\mathbf{\Delta A_{L\text{-}malic\ acid} = A_2 - (2 \times A_1)}$; (see Figure 1).

For example with values of $A_2 = 0.42$ and $A_1 = 0.02$, $\Delta A_{\text{L-malic acid}} = 0.42 - (2 \times 0.02) = 0.42 - 0.04 = 0.38$.

The value of $\Delta A_{\text{L-malic acid}}$ should as a rule be at least 0.10 absorbance units to achieve sufficiently accurate results.

The concentration of L-malic acid in the sample being analysed is determined using the equations below (derived from the standard curve in Figure 3, page 12):

Concentration of L-malic acid for PVPP treated samples.

with 20 µL sample volume and no sample dilution:

$$\mathbf{c = 0.771 \times \Delta A_{L\text{-}malic\ acid} \times 102/100} \quad [g/L]$$

$$\mathbf{= 0.786 \times \Delta A_{L\text{-}malic\ acid}} \quad [g/L]$$

with 20 µL sample volume and with sample dilution:

$$\mathbf{c = 0.786 \times dilution\ factor \times \Delta A_{L\text{-}malic\ acid}} \quad [g/L]$$

where:

102/100 = allowance for the dilution of wine by PVPP treatment.

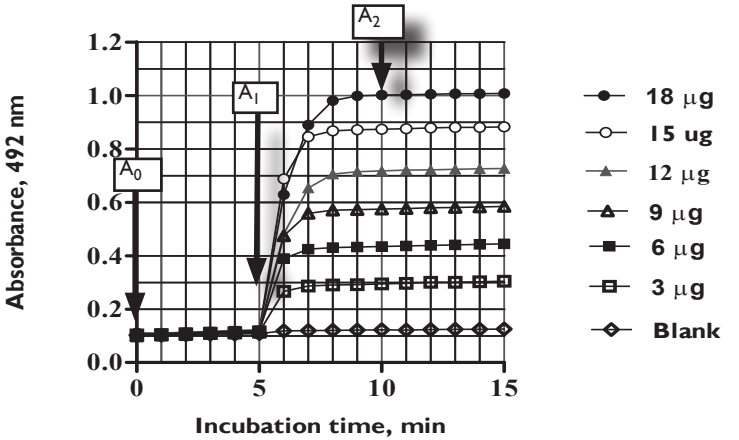

Figure 2. Time course of colour formation in the MegaQuant™ L-Malic Acid Test performed in a recording spectrophotometer (25°C, 1 cm light path, 492 nm). The sample analysed was a red wine following treatment with PVPP and filtration, and spiked with different quantities of L-malic acid (0-18 µg). On addition of the sample (A_0), the reaction was allowed to proceed for 5 min (A_1) to determine the rate of the creep reaction. L-Malate dehydrogenase (20 µL) was then added and the reaction was allowed to proceed for a further 10 min [the absorbance (A_2) is taken after 5 min]. When using the MegaQuant™ meter, the meter is zeroed when the tube is first inserted (thus A_0 is 0.00; see calculations on page 9).

SAMPLE PREPARATION:

Sample dilution.

The amount of L-malic acid present in the cuvette (i.e. in the 20 µL of sample being analysed) should range between 1.54 and 15 µg. The sample solution must therefore be diluted sufficiently to yield a L-malic acid concentration between 0.077 and 0.75 g/L.

Dilution Table

Estimated concentration of L-malic acid (g/L)	Dilution with water	Dilution factor (F)
< 0.75	No dilution required	1
0.75-7.5	1 + 9	10
7.5-75	1 + 99	100

SAMPLE PREPARATION EXAMPLES:

(a) Determination of L-malic acid in grape juice and white wine.

The free L-malic acid concentration of white wine and grape juice can generally be determined without any sample treatment (except dilution according to the dilution table). *Typically, no dilution (wine at the end of malolactic fermentation) or a dilution of 1:10 (grape juice) and sample volume of 20 µL are satisfactory.*

(b) Determination of L-malic acid in red wine.

Transfer approx. 5 mL of red wine to a 10 mL graduated polypropylene tube (equipment 2), using the graduations on the tube as a guide. Add one PVPP tablet, cap the tube and invert the contents continually over 5 min. The tablet will disintegrate after approx 1 min, but inversion of the tube must be continued intermittently for the full 5 min to get optimal binding of the phenolics. Filter the tube contents through a Whatman No. 1 (9 cm) filter circle and collect approx. 1 mL of filtrate. This solution must be no darker than a light pink colour, which it will be for most red wine samples. However, if the filtrate is still a deep red colour, repeat the treatment of the wine, but use two PVPP tablets. In such cases, to ensure that sufficient filtrate is collected, it is necessary to carefully squeeze the folded filter paper in the filter funnel with a metal spatula or tea spoon. An essentially clear, light pink coloured solution should be obtained, and this is suitable for analysis. *Typically, for red wine near the end of malolactic fermentation, no dilution is required and a volume of 20 µL is satisfactory.*

(c) Determination of L-malic acid in grapes.

Add 200 mL of distilled or filtered drinking water to a 500 mL measuring cylinder. Add a representative number of grapes to raise the volume to just over 400 mL. Add distilled water in a volume equivalent to the volume above 400 mL (e.g. if the measured volume is 420 mL, add another 20 mL of distilled water). This gives a 2-fold dilution of the volume of the grapes. Transfer the water plus grapes to a Waring blender (kitchen blender) and homogenise for approx 3 min. Filter an aliquot of this solution through a Whatman No. 1 filter sheet (9 cm diameter). Discard the first few mL and collect the next approx. 5-10 mL. Dilute the filtrate as required. *Typically, a further dilution of 10-fold (1 + 9 mL) and a sample volume of 20 µL are satisfactory (i.e. total sample dilution of 10-fold).*

Single grapes can be analysed by crushing the grape with a garlic press. Recover the juice in a 10 mL measuring cylinder and dilute with an equal volume of distilled water. Mix well and filter. Dilute the filtrate as required. *Typically, a further dilution of 10-fold (1 + 9 mL) and a sample volume of 20 µL are satisfactory (i.e. total sample dilution of 10-fold).*

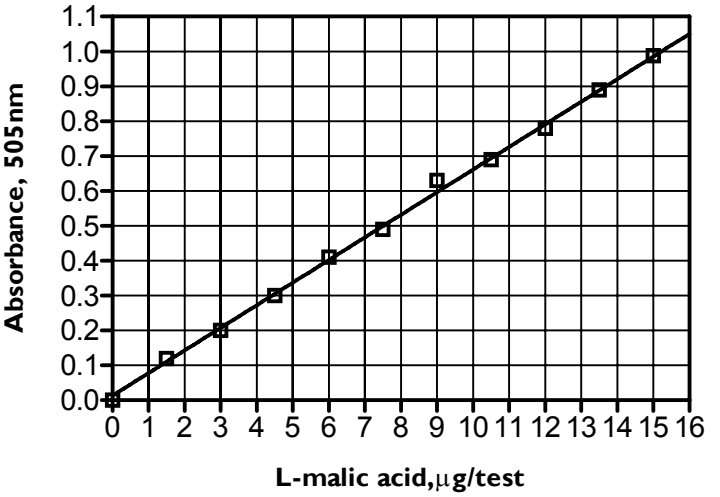

Figure 3. Standard curve relating L-malic acid concentration (µg/test) to absorbance measured with the MegaQuant™ colorimeter (at ambient temperature and 505 nm).

REFERENCES:

1. McCleary, B. V. & Charnock, S. J. (2005). Reagent components in tablet form for colorimetric assays of food and beverage analytes.

2. 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.

CHECKING THE ACCURACY AND RELIABILITY OF THE MegaQuant™ COLORIMETER AND THE L-MALIC ACID TEST:

1. Certified colour standards are available from Megazyme for checking the instrument calibration. Instrument calibration should not change in use.

2. Accuracy of the MegaQuant™ L-Malic Acid Test is determined using the provided L-malic acid standard. Perform an assay exactly as described on pages 5 and 6, using 20 µL of L-malic acid standard (0.375 mg/mL) in place of the sample. Allow the reaction to proceed to completion. The expected absorbance value (A_2-A_1) is 0.49. Values significantly different to this may be due to pipetting inaccuracies. If this possibility is excluded, contact Megazyme for advice.

GENERAL POINTS ON USE AND MAINTENANCE OF THE MegaQuant™ COLORIMETER:

Error Messages:

Various error messages may appear on the instrument display panel. The cause of the problem and the appropriate action is as follows:

Display	Cause	Action
E1	Coloured, dirty or scratched blank tube used.	Re-zero on a correct blank in a clean, dry tube.
E2 or E3	a) Incorrect blank used to zero instrument. b) Electrical fault in the instrument.	a) Re-zero instrument on a correct blank. b) Return instrument for repair.
E4	Battery voltage too low for correct reading.	Change batteries.
E5	Stray light has affected the reading.	a) Remove instrument from vicinity of spotlights or other bright light source. b) Check instrument case for damage.

Care and Maintenance:

Keep the test tubes clean. Place the plastic screw caps on the tubes before taking a reading to prevent test solutions being spilt into the instrument. Wipe off spillages and moisture immediately with a dry cloth. On no account should solvents or abrasive materials be used to clean the instrument.

Replace the batteries when the 'B' symbol appears on the display. To remove the battery compartment cover press gently on the sides of the cover and pull downwards. Use 2 x 1.5 V alkaline 'AA' batteries, MN 1500, LR6, E91, AM3 or equivalent. Remove batteries from the instrument if it is to be stored or left unused for a long period of time.

The MegaQuant™ colorimeter is guaranteed for a period of one year from the time of purchase excluding accidental damage or damage caused by unauthorised repair or misuse. Should repair be necessary, contact the Technical Services Department of Palintest Ltd. at www.palintest.com, alternatively phone: +44 (0) 191 491 0808, fax: +44 (0) 191 482 5372 or write to Palintest House, Kingsway, Team Valley, Tyne and Wear, NE11 0NS, UK, quoting the serial number shown in the battery compartment. This guarantee does not affect statutory rights.

Technical Specifications:

Instrument Type	Single beam colorimeter pre-programmed for absorbance readings at 505 nm.
Optics	Palintest M2 optical system with pulsed blue green LED, wavelength filter and photodetector.
Operating Range	0.00-1.20 absorbance units.
Operating Temperature	0-40°C/32-104°F.
Display	10 mm LCD.
Test Cells	For use with round, flat-bottomed, test tubes, 1.60 cm OD, 1.415 cm light path.
Operation	Single button.
Blank/Zero Setting	Optionally held in memory or reset for each reading.
Internal Calibration	Factory set. Recalibration through internal software.
Instrument Case	Splash-proof case with membrane key pad.
Power Supply	2 x 1.5 V batteries. Power management system with auto-switch off.
Size	Instrument only 17.3 x 7.5/4.4 x 4.1 cm.

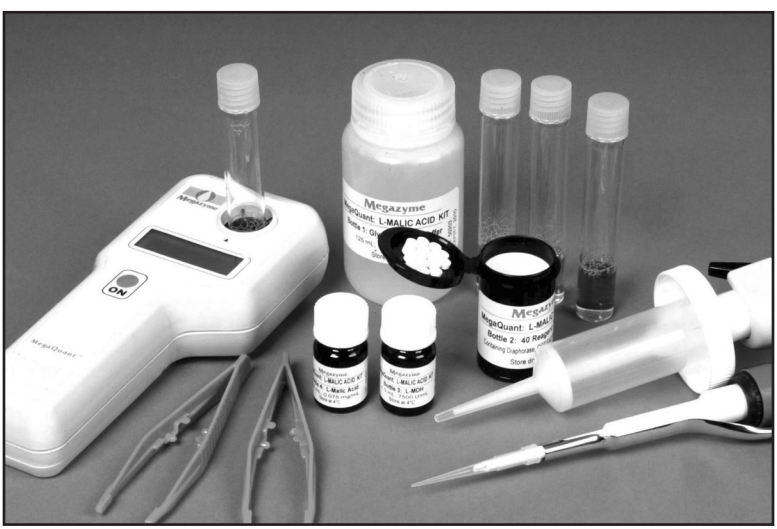

Figure 4. MegaQuant™ meter, tubes and reagents.

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