# L-MALIC ACID
# (LIQUID READY™)

PRODUCT INSTRUCTIONS

## SKU: 700007622
## K-LMLQR

04/24

(50 Manual Assays per Kit) or
(500 Auto-Analyzer Assays per Kit)

## 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.

## PRINCIPLE:

The detection of L-malic acid requires two enzymatic 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).

(1) L-Malic acid + $NAD^+$ $\xleftarrow{\text{(L-MDH)}}$ 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).

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

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

## SPECIFICITY, SENSITIVITY AND LINEARITY:

- The assay is specific for L-Malic Acid.
- The limit of detection (LOD) is 0.005 g/L, and the limit of quantification (LOQ) is 0.016 g/L (using a sample volume of 0.1 mL).
- The recommended measuring range is between 0.02 and 1.0 g/L (using a sample volume of 0.1 mL). This corresponds to 2 - 100 µg of L-Malic acid per assay.

## INTERFERENCE:

No interfering compounds have been identified.

## SAFETY:

The general safety measures that apply to all chemical substances should be adhered to. After use, the reagents can be disposed of with the laboratory waste.

**NOTE:** For more information regarding the performance of this product please refer to the associated validation report available from the Megazyme website. 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.

**KIT CONTENTS:**

Kits suitable for manual and automated format. The reagents are sufficient for performing 50 assays in manual format or 500 assays in auto-analyzer format. The kit contains:

**Reagent 1 (2 x 50 mL):** L-Glutamate
Contains sodium azide (0.05% *w/v*) as a preservative. Ready to use.
**Store at 4°C. See individual label for expiry date.**

**Reagent 2 (2 x 12.5 mL):** NAD, L-MDH and GOT
Contains sodium azide (0.05% *w/v*) as a preservative. Ready to use.
**Store at 4°C. See individual label for expiry date.**

**Standard (5 mL):** L-Malic Acid standard (1 g/L).
Contains sodium azide (0.05% *w/v*) as a preservative. Ready to use.
**Store at 4°C. See individual label for expiry date.**

**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.

**PREPARATION OF REAGENT SOLUTIONS:**

Bring all reagents to room temperature (20 - 25 °C) before use.

**MANUAL ASSAY PROCEDURE:**

**Wavelength:** 340 nm
**Cuvette:** 1 cm light path (glass or plastic)
**Temperature:** 20 - 37°C
**Final volume:** 2.60 mL
**Sample solution:** 0.02 g/L to 1 g/L (i.e. 2 - 100 µg of L-Malic Acid per cuvette)
**Read against air** (without a cuvette in the light path) or against water

| Pipette into Cuvettes | Blank | Sample |
|---|---|---|
| Reagent 1 | 2.0 mL | 2.0 mL |
| Sample | - | 0.1 mL |
| Distilled Water | 0.1 mL | - |
| Mix*, incubate for ~ 3 mins at 20 - 37°C, then read the absorbances ($A_1$)<br>Add Reagent 2 as described below: | | |
| Reagent 2 | 0.5 mL | 0.5 mL |
| Mix*, incubate for ~ 5 mins at 20 -37°C, then read the absorbances ($A_2$). ** | | |

* either by aspiration with the pipette tip used to dispense the liquid or by gentle inversion after sealing the cuvette with a cuvette cap or Parafilm®.
** It may be necessary to check if the reaction has reached completion by continuing to read the absorbances at 1

min intervals. If the reaction has not reached completion continue to measure absorbances until the values measured either remain the same, or increase constantly over 1 min. If this "creep" rate is greater for the sample than for the blank, extrapolate the absorbances (sample and blank) back to the time of addition of Reagent 2.

**NOTE:** The reagent blank value must be determined once for each run and subtracted from each sample result.

## CALCULATION:

**NOTE:** These calculations can be simplified by using the *MegaCalc*™ tool, downloadable from the product page.

### 1. Calculation of the dilution factor (df)

Determine the dilution factor (df) of the optical densities due to the reagent volume.

$$df = \frac{\text{Sample volume [mL]} + \text{R1 volume [mL]}}{\text{Total reaction volume [mL]}}$$

It follows for the L-Malic Acid manual assay procedure:

$$df = \frac{0.1 + 2.0}{2.6} = 0.808$$

### 2. Calculation of the absorbance difference $\Delta A_{\text{L-Malic Acid}}$

$$\Delta A_{\text{L-Malic Acid}} = (A_2 - df \times A_1)_{\text{sample}} - (A_2 - df \times A_1)_{\text{blank}}$$

It follows for the L-Malic Acid manual assay procedure:

$$\Delta A_{\text{L-Malic Acid}} = (A_2 - 0.808 \times A_1)_{\text{sample}} - (A_2 - 0.808 \times A_1)_{\text{blank}}$$

**NOTE:** Increasing or decreasing the sample volume with unchanged reagent volumes requires recalculation of the dilution factor; if volumes are changed, the system and performance may be affected.

### 3. Calculation of the L-Malic Acid content

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]

$\varepsilon$ = extinction coefficient of NADH at 340 nm [l x $mol^{-1}$ x $cm^{-1}$]

d = light path [cm]

v = sample volume [mL]

It follows for the L-Malic Acid manual assay procedure:

$$cc = \frac{2.6 \times 134.09}{6300 \times 1.0 \times 0.1} \times \Delta A_{\text{L-malic acid}} \quad [g/L]$$

$$= 0.5534 \times \Delta A_{\text{L-Malic Acid}} \quad [g/L]$$

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

**4. Calculation of the L-Malic Acid content in solid or semi-solid samples:**

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:

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

## AUTO-ANALYZER ASSAY PROCEDURE:

This kit has been designed for biochemistry analyzers and can be adapted to most instruments. A sample method is shown below (validated on the Awareness ChemWell®-T analyzer).

**NOTE:** For each batch of samples that is applied to the determination of L-Malic Acid a calibration curve must be performed concurrently using the same batch of reagents.

| **Parameter** | **Details** |
|---|---|
| Wavelength | 340/405 nm (primary/secondary) |
| Temperature | 20 - 37°C |
| Test | End-point test with following test sequence:<br>– Add Reagent 1 **[0.2 mL]**<br>– Add Sample or Calibrator **[0.01 mL]**<br>– Pre-incubate 1-3 min [20 - 37°C]<br>– Measure $A_1$ at 340/405 nm<br>– Add Reagent 2 **[0.05 mL]**<br>– Incubate 5 mins at [20 - 37°C]<br>– Measure $A_2$ at 340/405 nm<br>– Calculate $A_2 - A_1$ against calibration curve |
| Calibration | Calibrate using 2 – 4 calibrators ranging from 0 – 1.0 g/L.<br>The calibration curve is linear.<br><br>An example of how to use the standard supplied with the kit to create a calibration curve is shown below:<br>Calibrator 1 0 g/L (use distilled water)<br>Calibrator 2 0.1 g/L (dilute Standard 10-fold)<br>Calibrator 3 0.5 g/L (dilute Standard 2-fold)<br>Calibrator 4 1.0 g/L (use Standard as-is)<br>*Perform all dilutions with distilled water.* |

**SAMPLE PREPARATION:**

## 1. Sample dilution

The amount of L-Malic Acid present in the sample should range from 0.02 g/L to 1g/L. If the value of $\Delta A_{\text{L-Malic Acid}}$ is too low (e.g. <0.1), weigh more sample or decrease the dilution. If the value $\Delta A_{\text{L-Malic Acid}}$ is too high (e.g. >2.0), increase the dilution in distilled water.

**Dilution Table**

| Estimated Concentration of L-Malic Acid (g/L) | Dilution with Water | Dilution factor (F) |
|---|---|---|
| <1 | No Dilution required | 1 |
| 1 – 10 | 1mL sample + 9 mL water | 10 |
| 10 -100 | 1mL sample + 99 mL water | 100 |

## 2. General sample preparation guide

- Clear, slightly coloured and approximately neutral, liquid samples at a concentration up to 1.0 g/L can be used directly in the assay.
- Turbid samples should be filtered or centrifuged.
- Acidic samples (pH < 3.0) must be neutralised to approximately pH 8.0.
- Samples containing carbon dioxide should be degassed by gentle agitation or stirring with a glass rod.
- Solid samples should be homogenised, extracted in water and filtered or centrifuged if necessary.
- Strongly coloured samples should be treated by the addition of 0.2 g of polyvinylpolypyrrolidone (PVPP) per 10 mL of sample in a tube. Shake the tube vigorously for 5 minutes and then filter through filter paper.
- Deproteinise samples using the Megazyme Carrez Clarification Kit (K-CARREZ).
- Remove fat using Megazyme the Megazyme Carrez Clarification Kit (K-CARREZ).

## 3. Suggested sample preparation examples

**(a) Determination of L-Malic Acid in wine.** Pass through a 0.2 micron syringe filter to clarify. Alternatively, centrifuge an aliquot of wine for 5 minutes at 15,000 g. *Typically, a 3-fold dilution in distilled water is required.*

**(b) Determination of L-Malic Acid in fruit juice (e.g. apple juice).** Pass through a 0.2 micron syringe filter to clarify. Alternatively, centrifuge an aliquot of juice for 5 minutes at 15,000 g. *Typically, a 50-fold dilution in distilled water is required.*

**(c) Determination of L-Malic Acid in beer (e.g. Lager).** Remove carbonation by stirring a sample in a beaker for approximately 60 seconds using a glass rod. Pass through a 0.2 micron syringe filter and use the clear filtrate in the assay. *Typically, no dilution is required.*

**(d) Determination of L-Malic Acid in confectionary products (e.g. sour jellies).** Accurately weigh 4 g of confectionary into a 100 mL graduated cylinder and adjust to 100 mL with distilled water. Quantitatively transfer the contents of the cylinder to a blender and blend for 30 seconds, or until homogeneous. Allow any foam formed to settle and the liquid can then be used in the assay protocol with no further treatment. *Typically, no dilution is required.*

**(e) Determination of L-Malic Acid in fruit and vegetables (e.g. Broccoli).** Homogenize solid samples in a blender. Weigh 5 g of broccoli into a beaker and extract with 70 mL of distilled water heated to 60 °C. Quantitatively transfer into a 100 mL volumetric flask. Fill up to the mark with distilled water, mix, filter using filter a paper filter and use the clear filtrate in the assay. *Typically, no dilution is required.*

**IMPORTANT NOTE:** Users should perform in-house matrix validation prior to routine use. This process will highlight any problematic matrices encountered. The above are suggested sample preparation examples only. If you have questions about these or other matrices, please contact your local sales representative for support.

## SERVICES AND TECHNICAL SUPPORT

Please reach out to your local sales representative should you require any assistance, particularly in relation to:

- Troubleshooting
- Data analysis
- Additional matrix testing
- Application support in relation to automated analyzers

Supporting documents can be found in the product page:

- Quick Reference Guide
- MegaCalc™
- Safety Data Sheets (SDS)
- Certificates Of Analysis (COA)
- Validation Report

Contact us for more information: neogen.com/contact

**Without guarantee**

The information contained in this assay protocol is, to the best of our knowledge, true and accurate, but since the conditions of use are beyond our control, no warranty is given or is implied in respect of any recommendation or suggestions which may be made or that any use will not infringe any patents. It is the user's responsibility to perform in-house matrix validation work prior to routine use.