L-MALIC ACID
(LIQUID READY)

PRODUCT INSTRUCTIONS

SKU: 700004313
K-LMALQR

07/24

(1100 Auto-Analyser Assays per Kit) or
(1100 Microplate Assays per Kit)

INTRODUCTION:

As a component of the citric acid cycle, L-malic acid (L-malate) is found in all living organisms. The quantitative determination of L-malic acid 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 kit (**K-LMALQR**) is suitable for the specific measurement of L-malic acid in wines, beverages, foodstuffs and other materials.

PRINCIPLE:

(1) L-Malic acid + NAD^+ —(L-malate dehydrogenase; L-MDH)→ oxaloacetate + NADH

(2) Oxaloacetate + L-glutamate —(glutamate-oxaloacetate transaminase; GOT)→ 2-oxoglutarate + L-aspartate

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 1100 assays in auto-analyser format or microplate format are available from Neogen. The kits contain the full assay method plus:

Bottle 1:	**Reagent 1 (44 mL)** Contains sodium azide (0.08% w/v) as a preservative. Ready to use. Store at 4°C. See individual label for expiry.
Bottle 2:	**Reagent 2 (22 mL)** Contains sodium azide (0.05% w/v) as a preservative. Ready to use. Store at 4°C. See individual label for expiry.
Bottle 3:	**L-Malic Acid Standard (2 mL)** (6 g/L). Ready to use. Store sealed at 4°C. See individual label for expiry.

ASSAY PROCEDURES:

A. AUTO-ANALYSER FORMAT:

NOTE: 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
Calculation: End-point
Temperature: ~ 25°C or 37°C
Reaction: Absorbance increase
Final volume: 0.223 mL
Linearity: 0.5-18 µg of L-malic acid per cuvette
(in 0.003 mL sample volume)
(up to 6000 mg/L of original sample)

Pipette into cuvettes	Sample	Standard
reagent 1	0.040 mL	0.040 mL
distilled water	0.160 mL	0.160 mL
sample	0.003 mL	-
standard	-	0.003 mL
Mix, read the absorbances of the solutions (A_1) after 3 min and start the reactions by addition of:		
reagent 2	0.020 mL	0.020 mL
Mix and read the absorbances of the solutions (A_2) after 3 min.		

CALCULATION FORMULA:

$A_2 - A_1$

B. MICROPLATE FORMAT:

NOTE: 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 or 37°C
Final volume: 0.223 mL
Linearity: 0.5-18 µg of L-malic acid per cuvette
(in 0.003 mL sample volume)
(up to 6000 mg/L of original sample)

Pipette into well	Sample	Standard	Blank
reagent 1	0.040 mL	0.040 mL	0.040 mL
distilled water	0.160 mL	0.160 mL	0.163 mL
sample	0.003 mL	-	-
standard	-	0.003 mL	-
Mix* and read absorbances of the solutions (A_1) after 3 min. **Then add:**			
reagent 2	0.020 mL	0.020 mL	0.020 mL
Mix* and read the absorbances of the solutions (A_2) at the end of the reaction (approx. 3 min).			

* for example using microplate shaker, shake function on a microplate reader or repeated aspiration using a pipettor.

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.