Megazyme

L-MALIC ACID (L-MALATE)

(Liquid Stable Format)

ASSAY PROCEDURE

K-LMALQR 09/11

(1100 Auto-Analyser 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 reagents used in the determination of L-malic acid are not hazardous materials in the sense of the Hazardous Substances Regulations. However, the buffer concentrates contain sodium azide (up to 0.08 % w/v) as a preservative. The general safety measures that apply to all chemical substances should be adhered to.

KITS:

Kits suitable for performing 1100 assays in auto-analyser format are available from Megazyme. 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.
Stable for > 2 years at 4°C.

Bottle 2: **Reagent 2 (22 mL)**
Contains sodium azide (0.05 % w/v) as a preservative.
Ready to use.
Stable for > 18 months at 4°C.

Bottle 3: **L-Malic Acid Standard (2 mL)**
(6 g/L). Ready to use.
Stable for > 2 years at room temperature.

ASSAY PROCEDURES:

A. AUTO-ANALYSER FORMAT:

NOTE: For each batch of samples that are 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: 0.5-14 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, read the absorbances of the solutions (A_2) after 3 min.		

CALCULATION FORMULA:

$A_2 - (A_1 \times 203 / 223)$

B. MICROPLATE FORMAT:

NOTE: For each batch of samples that are 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: 0.5-14 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*, read absorbances of the solutions (A_1) after 3 min. Then add:			
reagent 2	0.020 mL	0.020 mL	0.020 mL
Mix*, 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.

Megazyme

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