L-MALIC ACID AUTO

125 ml

INTENDED USE

Reagent for enzymatic determination of L-malic acid in foodstuff and other sample material.

PRINCIPLE OF THE METHOD

Malic acid (L-malate) is oxidized to Oxaloacetate by Nicotinamide-Adenine Dinucleotide (NAD) in the presence of the enzyme L-Malate Dehydrogenase (L-MDH), yielding NADH. The equilibrium of the reaction is shifted towards L-Malate. The enzyme Glutamate-Oxaloacetate Transaminase (GOT) in the presence of L-Glutamate, subtracts Oxaloacetate from the reaction medium by catalysing its conversion to L-Aspartate, with formation of 2-Oxoglutarate.
NADH is produced in stoichiometrical concentration with respect to L-Malate. Increase of NADH is measured photometrically at 340 nm.

KIT COMPONENTS

The components of the kit are stable until expiration date on the label.
Keep away from direct light sources.

L-MAL AUTO R1: 2 x 50 ml (liquid) blue cap
Composition: Buffer pH 9.5, Glutamate 13 mM, GOT ≥ 1 KU/l, MDH ≥ 1 KU/l, preservatives.

L-MAL AUTO R2: 1 x 25 ml (liquid) red cap
Composition: Buffer pH 7.0, NAD^+ 20 mM, preservatives.

Store all components at 2-8°C.

In vitro use only.

MATERIALS REQUIRED BUT NOT SUPPLIED

Current laboratory instrumentation. Spectrophotometer UV/VIS with thermostatic cuvette holder. Automatic micropipettes. Glass or high quality polystyrene cuvettes. Standard solution.
Multiparametric Standard (code SQPE053234) is available on request. Please contact customer service for further information.

REAGENT PREPARATION

Use separate reagents.
Stability: until expiration date on the label at 2-8°C.

Preparation of standard 1 g/l:
Dilute multiparametric standard 5 g/l (SQPE053234) 1:5 with distilled water (1 part of standard and 4 parts of water), thus obtaining a standard of concentration 1 g/l.

PRECAUTIONS

L-MAL AUTO R1: Warning. Causes serious eye irritation (H319). Causes skin irritation (H315). Wear protective gloves. Eye protection (P280). IF ON SKIN: Wash with plenty of water (P302+P352). IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing (P305+P351+P338). If eye irritation persists: get medical advice (P337+P313).

L-MAL AUTO R2: It is not classified as hazardous.

SPECIMEN

Wine or any foodstuff once its utilization has been tested. Red wines with total polyphenols > 3.0 g/l should be decoloured with activated charcoal or PVPP 1% w/v. Taking into account the process of malo-lactic refermentation, it is suggested a 1:5 dilution of the sample (4 ml of deionized water + 1 ml of sample), then multiply by 5.
When esterified L-malic acid is to be determined as well, the sample should be treated as follows:
add 6 ml of 2M NaOH to 20 ml of wine, heat under reflux for 30 min in a flask. After cooling to room temperature, neutralize the solution with 1 M sulfuric acid by the use of a litmus-paper. Transfer the liquid in a volumetric flask and adjust the volume to 50 ml. Analyze the sample according to the general procedure and multiply the result by the dilution factor (2.5). The result is the sum of the free and esterified L-malic acid.

PROCEDURE

Wavelength: 340 nm
Lightpath: 1 cm
Temperature: 37°C

dispense:	blank	standard	sample
reagent R1	2 ml	2 ml	2 ml
water	50 µl	-	-
standard	-	50 µl	-
sample	-	-	50 µl

Mix, incubate at 37°C for 3 minutes.
Read absorbances of standard (As_1) and sample (Ac_1) against reagent blank.

dispense:	blank	standard	sample
reagent R2	500 µl	500 µl	500 µl

Mix, incubate at 37°C for 10 minutes.
Read absorbances of standard (As_2) and sample (Ac_2) against reagent blank.

RESULTS CALCULATION

$$\text{Malic acid g/l} = \frac{Ac_2 - (0.8 \times Ac_1)}{As_2 - (0.8 \times As_1)} \times \text{standard value}$$

TEST PERFORMANCE

Specificity
The method is specific for L-malic acid.

Linearity
The method is linear up to 2.5 g/l.
If the limit value is exceeded, it is suggested to dilute the sample 1+4 with distilled water and to repeat the test, multiplying the result by 5.

Precision
White wine

intra-assay (n=10)	mean (g/l)	SD (g/l)	CV%
sample	1.620	0.013	0.80

inter-assay (n=20)	mean (g/l)	SD (g/l)	CV%
sample	1.605	0.051	3.18

Rose wine

intra-assay (n=10)	mean (g/l)	SD (g/l)	CV%
sample	0.710	0.013	1.81

inter-assay (n=20)	mean (g/l)	SD (g/l)	CV%
sample	0.718	0.013	2.19

WASTE DISPOSAL

This product is made to be used in professional laboratories.
P501: Dispose of contents according to national/international regulations.

REFERENCES

H.U.Bergmeyer ed. 3, "Methods of enzymatic analysis" vol. VII
UNI EN 1138:1997 "Determinazione enzimatica del contenuto di acido L-malico (L-malato). Metodo spettrometrico con NADH"

MANUFACTURER

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SYMBOLS


lot of manufacturing


code number


storage at temperature interval


expiration date (year/month)


warning, read enclosed documents


read the directions