L-MALIC ACID

5 x 20 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 dehydrogenase (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 R1: 5 x 20 ml (liquid) blue cap
Composition: Glycylglycine buffer pH 9.00, GOT ≥ 600 U/l, MDH ≥ 1 KU/l, glutamate 13 mM, $MgCl_2$ 8 mM, preservatives.

L-MAL R2: 1 x 2.5 ml (liquid) white cap
Composition: TRIS pH 7.00, NAD 150 mM, preservatives.

L-MAL BL: 2 x 50 ml (liquid) white cap
Composition: Glycylglycine buffer pH 9.00, 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

Procedure 1:
Use separate reagents.
Stability: until expiration date on the label at 2-8°C.

Procedure 2:
Working reagent: mix 40 parts of reagent R1 with 1 part of reagent R2.
It is suggested to prepare strictly the amount needed for the analysis, and any residue has to be stored at 2-8°C away from direct light sources.
Analytical performances of mixed reagent begin to fall off 48 hours after its preparation.

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 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 R2: It is not classified as hazardous.

L-MAL BL: 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 1

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	50 µl	50 µl	50 µl

Mix, incubate at 37°C for 5 minutes.
Read absorbances of standard (As_2) and sample (Ac_2) against reagent blank.

RESULTS CALCULATION

$$\text{Malic acid g/l} = \frac{Ac_2 - Ac_1}{As_2 - As_1} \times 1 \text{ (standard value)}$$

PROCEDURE 2 (cell flow instruments)

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

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

Mix, incubate at 37°C for 5 minutes.
Read absorbances of standard (As_1), sample (Ac_1), reagent blank (Ar_1) and sample blank (Ac_2).

RESULTS CALCULATION

$$\text{Malic acid g/l} = \frac{(Ac_1 - Ar_1) - Ac_2}{As_1 - Ar_1} \times 1 \text{ (standard value)}$$

TEST PERFORMANCE

Specificity
The method is specific for L-malic acid.

Linearity
The method is linear up to 1.25 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=9)	mean (g/l)	SD (g/l)	CV%
sample	1.28	0.01	0.80
inter-assay (n=20)	mean (g/l)	SD (g/l)	CV%
sample	1.25	0.04	2.77

Red wine

intra-assay (n=8)	mean (g/l)	SD (g/l)	CV%
sample	0.09	0.004	4.19
inter-assay (n=21)	mean (g/l)	SD (g/l)	CV%
sample	0.08	0.004	5.49

Rose wine

intra-assay (n=10)	mean (g/l)	SD (g/l)	CV%
sample	0.50	0.01	0.96
inter-assay (n=20)	mean (g/l)	SD (g/l)	CV%
sample	0.52	0.02	3.33

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 lot of manufacturing
REF code number
storage at temperature interval
expiration date (year/month)
warning, read enclosed documents
read the directions