D-MALIC ACID

5 x 25 ml

INTENDED USE

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

PRINCIPLE OF THE METHOD

The enzyme D-Malate dehydrogenase (D-MDH) catalizes the selective oxidation of D-Malic Acid, in the presence of NAD. L-Malic Acid, the naturally occurring isomer which is present in solution, does not undergo reaction. Absorbance increase due to NADH formation, measured at 340 nm, is proportional to the amount of D-Malic Acid of the sample.

KIT COMPONENTS

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

D-MAL R1A: **5 x 20 ml (liquid) blue cap**
Composition: Buffer, stabilizers and preservatives

D-MAL R1B: **1 x 25 ml (liquid) red cap**
Composition: Buffer, NAD^+ ≥ 10 mM, preservatives

D-MAL R2: **1 x 2.5 ml (liquid) white cap**
Composition: Suspension of D-MDH

D-MAL BL: **2 x 62.5 ml (liquid) white cap**
Composition: Buffer, 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.
Muliparametric standard code SQPE053234 is available on request. Please contact customer service for further information.

REAGENT PREPARATION

Reagent R1: mix 4 parts of reagent R1A with 1 part of reagent R1B.
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.
Reagent R1 is stable at least 14 days since its preparation.

Procedure 1:
Use separate R1 and R2 reagents.
Since preparation of Reagent R1, the calibration is stable at least for 10 days, with periodical update of reagent blank.

Procedure 2:
Working reagent: mix 50 parts of reagent R1 with 1 part of reagent R2 (gently shake the vial prior to use).
Attention: analytical performances of mixed reagent begin to fall off quickly, so it is suggested to prepare strictly the amount needed and set up the assay in the machine in batch mode.

Procedure 3:
Use separate R1A, R1B and R2 reagents.

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

D-MAL R1A: 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).

D-MAL R1B: It is not classified as hazardous.

D-MAL R2: It is not classified as hazardous.

D-MAL BL: 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).

SPECIMEN

Wine or any foodstuff once its utilization has been tested.
Red wines should be decoloured with activated charcoal or PVPP 1% w/v.

PROCEDURE 1

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

dispense:	reagent blank	standard	sample
reagent R1	2 ml	2 ml	2 ml
water	20 µl	-	-
standard	-	20 µl	-
sample	-	-	20 µl

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

dispense:	reagent blank	standard	sample
reagent R2	40 µl	40 µl	40 µl

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

RESULTS CALCULATION

$$\text{D-Malic Acid g/l} = \frac{Ac_2 - Ac_1}{As_2 - As_1} \times \text{standard value}$$

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	20 µl	-	-	-
standard	-	20 µl	-	-
sample	-	-	20 µl	20 µl
blank	-	-	2 ml	-

Mix, incubate at 37°C for 10 minutes.
Read absorbances of standard (As_1), sample (Ac_1), reagent blank (Ar_1) and sample blank (Ac_2).

RESULTS CALCULATION

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

PROCEDURE 3

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

dispense:	reagent blank	standard	sample
reagent R1A	2 ml	2 ml	2 ml
reagent R1B	500 µl	500 µl	500 µl
water	25 µl	-	-
standard	-	25 µl	-
sample	-	-	25 µl

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

dispense:	reagent blank	standard	sample
reagent R2	50 µl	50 µl	50 µl

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

RESULTS CALCULATION

$$\text{D-Malic Acid g/l} = \frac{Ac_2 - Ac_1}{As_2 - As_1} \times \text{standard value}$$

TEST PERFORMANCE

Specificity
The method is specific for D-malic acid.

Linearity
The method is linear up to 1 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	0.411	0.009	2.23
inter-assay (n=20)	mean (g/l)	SD (g/l)	CV%
sample	0.411	0.020	4.92

Red wine

intra-assay (n=10)	mean (g/l)	SD (g/l)	CV%
sample	0.538	0.007	1.26
inter-assay (n=20)	mean (g/l)	SD (g/l)	CV%
sample	0.538	0.030	5.62

Rose wine

intra-assay (n=10)	mean (g/l)	SD (g/l)	CV%
sample	0.449	0.006	1.29
inter-assay (n=20)	mean (g/l)	SD (g/l)	CV%
sample	0.457	0.023	5.01

WASTE DISPOSAL

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

REFERENCES

Beutler, Ara *Fruit Processing* 1992, 2, 140

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

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