D-LACTIC ACID

5 x 20 ml

INTENDED USE

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

PRINCIPLE OF THE METHOD

D-lactic acid (D-lactate) is oxidised to pyruvate in the presence of D-lactate dehydrogenase (D-LDH) and nicotinamide-adenine dinucleotide (NAD+). The equilibrium of such a reaction is far in favour of lactate. However, lactate is dehydrogenated completely by LDH if pyruvate is withdrawn from the equilibrium by conversion to alanine by means of the enzyme D-glutamate-pyruvate transaminase (D-GPT). This reaction functions as a trapping enzymatic reaction for pyruvate.
The amount of NADH formed in the above coupled reaction is stoichiometric with the amount of D-lactic acid. It is the NADH which is measured by the increase in absorbance at 340 nm.

KIT COMPONENTS

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

D-LAT R1: 5 x 20 ml (liquid) blue cap
Composition: Buffer pH 7.00, GPT ≥ 600 U/l, D-LDH ≥ 5 KU/l, NAD 150 mM , $MgCl_2$ 8 mM, preservatives.

D-LAT R2: 1 x 2.5 ml (liquid) white cap
Composition: Buffer pH 12.00, glutamate 13 mM, preservatives.

D-LAT BL: 2 x 50 ml (liquid) white cap
Composition: Glycylglycine buffer pH 9.50, 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 0.6 g/l:
dilute multiparametric standard 3 g/l (SQPE053234) 1:5 with distilled water (1 part of standard and 4 parts of water), thus obtaining a standard of concentration 0.6 g/l.

PRECAUTIONS

D-LAT R1: It is not classified as hazardous.

D-LAT R2: 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-LAT 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.

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 15-20 minutes.
Read absorbances of standard (As_2) and sample (Ac_2) against reagent blank.

RESULTS CALCULATION

$$\text{D-Lactic acid g/l} = \frac{Ac_2 - Ac_1}{As_2 - As_1} \times 0.6 \text{ (standard value)}$$

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 15-20 minutes.
Read absorbances of standard (As_1), sample (Ac_1), reagent blank (Ar_1) and sample blank (Ac_2).

RESULTS CALCULATION

$$\text{D-Lactic acid g/l} = \frac{(Ac_1 - Ar_1) - Ac_2}{As_1 - Ar_1} \times 0.6 \text{ (standard value)}$$

TEST PERFORMANCE

Specificity
The method is specific for D-Lactic acid.

Linearity
The method is linear up to 0.6 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	0.203	0.004	1.790
inter-assay (n=21)	mean (g/l)	SD (g/l)	CV%
sample	0.202	0.005	2.564

Red wine

intra-assay (n=10)	mean (g/l)	SD (g/l)	CV%
sample	0.365	0.004	1.041
inter-assay (n=20)	mean (g/l)	SD (g/l)	CV%
sample	0.359	0.007	1.967

Rose wine

intra-assay (n=10)	mean (g/l)	SD (g/l)	CV%
sample	0.237	0.004	1.690
inter-assay (n=21)	mean (g/l)	SD (g/l)	CV%
sample	0.235	0.005	2.045

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. VI
Recueil des methodes internationales d’analyse des vins et des mouts

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