D-/L-Lactic acid, UV method

Catalogue number: AK00141, 50 tests of each

Application

This rapid and simple stereo-specific enzymatic method is used for the determination of D-(-)-lactic acid (D-(-)-lactate) and L-(+)-lactic acid (L-(+)-lactate) in foodstuffs such as milk and milk products (e. g. cheese, yogurt), wine, beer, dietetic food, fruit and vegetable products (e. g. juices, jam, tomato pulp), meat products, soft drinks and lemonades, vinegar, as well as in cosmetics, paper and cardboard, pharmaceuticals and biological samples. See also our test kits for D-lactic acid (cat. AK00121) and L-lactic acid (cat. AK00131).

Introduction

D-Lactic acid is a natural product formed only by lactic acid bacteria, such as *Lactobacillus lactis*, *Lactobacillus bulgaricus* and *Leuconostoc cremoris*. L-Lactic acid is a common final product of the metabolism of a wide variety of living organisms, including lactic acid bacteria. L-Lactate in wine is also formed during the malo-lactic fermentation ("second fermentation"). The production of D-lactic acid can indicate wine spoilage. The content of L-lactate in beer indicates the presence of *Lactobacilli* in production. Similarly, the quality of milk and fruit juice can be established by measurement of the D- and L-lactic acid content. L-Lactic acid is supplemented into foods and beverages (E270) where a tart flavour is desired, and is widely used as a non-volatile acidulant. The stereo-specific measurement of the lactate forms is of high interest *e.g.* in the manufacturing of sour milk products in order to assess the activity of microorganisms. The presence of D-lactic acid may indicate a microbial contamination. The content of L-lactate in liquid whole egg or in egg powder gives good information about the hygienic situation of the products.

Principle

Although the assays for D-lactic and L-lactic acids can be performed sequentially, in the current kit they are performed separately since this allows the incubations to be performed in parallel which reduces the total reaction time. The determination of D-lactic acid requires the following two coupled reactions:

$$\text{D-Lactate} + \text{NAD}^+ \xrightarrow{\text{D-LDH}} \text{Pyruvate} + \text{NADH} + \text{H}^+$$

$$\text{Pyruvate} + \text{D-Glutamate} \xrightarrow{\text{D-ALT}} \text{D-Alanine} + \text{2-Oxoglutarate}$$

The amount of NADH formed through the combined action of D-lactate dehydrogenase (D-LDH; EC 1.1.2.3) and D-alanine aminotransferase (D-ALT/D-GPT; EC 2.6.1.2) is measured at 340 nm. Since the first reaction is an equilibrium reaction, a coupled one is necessary to combine in order to complete the reaction (endpoint analysis).

The determination of L-lactic acid requires a similar set of reactions (also endpoint analysis) but the oxidation to pyruvate by nicotinamide-adenine dinucleotide (NAD+) is catalyzed by L-lactate dehydrogenase (L-LDH), as follows:

$$\text{L-Lactate} + \text{NAD}^+ \xrightarrow{\text{L-LDH}} \text{Pyruvate} + \text{NADH} + \text{H}^+$$

Specificity

The determination is specific for D- and L-lactic acids.

Sensitivity and detection limit

The sensitivity of the assay is based on 0.005 AU and a sample volume of 1.50 mL. This corresponds to a D- and L-lactic acids concentration of 0.107 mg/L sample solution when measured at 340 nm. For both acids, the detection limit of 0.214 mg/L results from the absorbance difference of 0.010 (340 nm) and a maximum sample volume of 1.50 mL.

Linearity and precision

Linearity of the determination exists from 0.5 to 30 µg D-lactic acid, and from 0.3 to 30 µg L-lactic acid, per assay (v = 1.50 mL). For both acids, in a double assay using one sample solution, a difference of 0.005 to 0.010 AU may occur (0.107-0.214 mg/L of D-/L-lactic acid, v = 1.50 mL). The CV is approx. 1 to 3% in the measuring range.

Kit composition

Solution 1 (×2). Glycylglycine buffer (25 mL, 0.5 M, pH 10.0), D-glutamate (0.5 M) and sodium azide (0.02% w/v) as a preservative. Stable for 2 years at 4 °C.

Solution 2 (×2). NAD^+ (380 mg) and PVP (60 mg). Stable for 5 years at -20 °C.

Dissolve in 5.5 mL of distilled water, divide into appropriately sized aliquots and store in PP tubes at -20 °C between use (stable for 2 years) and keep cool during use. Do not dissolve the content of the second bottle until required. Once dissolved, the reagent is stable for 2 years at -20 °C.

Suspension 3. D-Alanine aminotransferase (D-ALT, 1300 U/mL) in 3.2 M ammonium sulphate (1.1 mL). Stable for 2 years at 4 °C.

Suspension 4L. L-Lactate dehydrogenase (L-LDH, 2000 U/mL) in 3.2 M ammonium sulphate (1.1 mL). Stable for 2 years at 4 °C.

Suspension 4D. D-Lactate dehydrogenase (D-LDH, 2000 U/mL) in 3.2 M ammonium sulphate (1.1 mL). Stable for 2 years at 4 °C.

Solution 5. D-/L-Lactic acids standard solution (5 mL, 0.15 mg/mL of each acid). Stable for 2 years at 4 °C.

This standard can be used when there is doubt about the method accuracy ($\varepsilon_{NADH,340\ nm}$ = 6300 $L \times mol^{-1} \times cm^{-1}$).

Safety

Reagents that are used in the determination of D- and L-lactic acids are not hazardous materials (see Hazardous Substances Regulations). However, the general safety measures that apply to all chemical substances should be followed.

Procedure for D-Lactic acid (endpoint analysis)

Wavelength: 340 nm

Cuvette: 1 cm light path (glass or plastic)

Temperature: ~ 25 °C

Final volume: 2.24 mL

Sample solution: 0.5-30 µg of D-lactic acid per cuvette (in 0.10-1.5 mL sample volume)

Read against air (without a cuvette in the light path) or against water

Pipette into cuvettes (mL)	**Blank**	**Sample**
Distilled water (at ~25 °C)	1.60 mL	1.50 mL
Sample	-	0.10 mL
Solution 1 (glycylglycine buffer)	0.50 mL	0.50 mL
Solution 2 (NAD^+)	0.10 mL	0.10 mL
Suspension 3 (D-ALT)	0.02 mL	0.02 mL
Mix, measure the absorbance of the solutions (A1) after ~3 min and start the reaction by addition of		
Suspension 4D (D-LDH)	0.02 mL	0.02 mL
Mix, measure the absorbance of the solutions (A2) at the end of the reaction (approx. 5 min)*		

Mixtures can be obtained with a plastic spatula or by gentle inversion after sealing with a cuvette cap or Parafilm®.

* If the reaction has not stopped after 5 min, continue measuring absorbance until the absorbance either remain the same, or increase constantly over 1 min. If this "creep" rate is greater for the sample than for the blank, extrapolate the absorbance (sample and blank) back to the time of addition of suspension 4D.

Procedure for L-Lactic acid (endpoint analysis)

Wavelength: 340 nm

Cuvette: 1 cm light path (glass or plastic)

Temperature: ~ 25 °C

Final volume: 2.24 mL

Sample solution: 0.3-30 µg of L-lactic acid per cuvette (in 0.10-1.5 mL sample volume)

Read against air (without a cuvette in the light path) or against water

Pipette into cuvettes (mL)	**Blank**	**Sample**
Distilled water (at ~25 °C)	1.60 mL	1.50 mL
Sample	-	0.10 mL
Solution 1 (glycylglycine buffer)	0.50 mL	0.50 mL
Solution 2 (NAD^+)	0.10 mL	0.10 mL
Suspension 3 (D-ALT)	0.02 mL	0.02 mL
Mix, measure the absorbance of the solutions (A1) after ~3 min and start the reaction by addition of		
Suspension 4L (L-LDH)	0.02 mL	0.02 mL
Mix, measure the absorbance of the solutions (A2) at the end of the reaction (approx. 10 min).*		

Mixtures can be obtained with a plastic spatula or by gentle inversion after sealing with a cuvette cap or Parafilm®.

*If the reaction has not stopped after 10 min, continue measuring absorbance until the absorbance either remain the same, or increase constantly over 5 min. If this "creep" rate is greater for the sample than for the blank, extrapolate the absorbance (sample and blank) back to the time of addition of suspension 4L.

Calculation

Determine the absorbance differences for both blank and sample (A2-A1). The concentration of D-lactic acid (g/L), or L-lactic acid (g/L), based on the ε of NADH at 340 nm (6300 $L \times mol^{-1} \times cm^{-1}$), is calculated as follows:

$$C\ (D\text{-}/L\text{-lactic acid}) = 0.3204 \times \Delta A_{D\text{-lactic acid}/\ L\text{-lactic acid}}$$

If the sample has been diluted or a different sample volume was used during the reaction, the result must be multiplied by the corresponding dilution/concentration factor.

Alternative procedures (micro-volumes)

Although this kit has been developed to work in cuvettes, it can be easily adapted for use in 96-well microplates or in auto-analysers. Basically, the assay volumes for the cuvette format have to be reduced approximately 10-fold for use in microplate format or in auto-analyser format. However, when using these micro-volume formats, you must be aware that the radiation pathlength is usually smaller than 1 cm, which is the standard cuvettes pathlength. Thus, to perform the calculation of the amount of analyte in the samples follow one of the three possible strategies described in the "Alternative Procedures Brochure", available on the NZYTech website.

Interferences

If the conversion of D-lactic acid and L-lactic acid have been completed within the time specified in the assay (approx. 5 min and 10 min, respectively), no interference has occurred. However, this can be further checked by adding D-lactic acid (approx. 15 µg in 0.1 mL) or L-lactic acid (approx. 15 µg in 0.1 mL) to the cuvette on completion of the reaction. A significant increase in the absorbance should be observed.

Perspiration of the hands contains L-lactic acid, thus care should be taken not to touch the tips of the pipettes.

Interfering substances in the sample can be identified by the use of D-lactate or L-lactate as internal standard. Quantitative recovery of this standard are expected.

General information on sample preparation

The amount of D- and L-lactic acids present in the cuvette should range between 0.5 and 30 µg, and 0.3 and 30 µg, respectively. Thus, if a sample volume of 0.10 mL is used the sample solution must be diluted to yield D- and L-lactic acids concentrations between 5 and 300 mg/L, and 3 and 300 mg/L, respectively. However, the sample volume can range from 0.10 to 1.50 mL, by replacing water (D- and L-lactic acids range from 0.33 to 300 mg/L and 0.20 to 300 mg/L, respectively).

Polyvinylpyrollidone (PVP) has been incorporated into the assay in order to prevent the interference from particular tannins found especially in red wine.

To implement this assay use clear, colourless liquid samples, with pH adjusted to 10.0, directly, or after dilution. Filter turbid solutions; degas samples containing carbon dioxide (*e.g.* by filtration); adjust acid samples, which are used undiluted for the assay, to pH 10.0 by adding sodium or potassium hydroxide solution and incubate at room temperature for approx. 30 min; measure "coloured" samples (if necessary, adjust to pH 10.0) against a sample blank (i.e. sample without L-LDH); treat "strongly coloured" samples that are used undiluted or with a higher sample volume with PVPP (add 0.2 g of PVPP/10mL of sample, shake for 5 min and filter using Whatman No. I filter paper); crush or homogenize solid or semi-solid samples, extract with water or dissolve; extract samples containing fat with hot water.

Examples of sample preparation

Determination of free and esterified D- and L-lactic acids in white and red wines

The free D-/L-lactic acids concentration of white and red wines can usually be quantified without any sample treatment. Typically, a dilution of 1:10 and a sample volume of 0.1 mL are appropriate.

The concentration of both free and esterified D-/L-lactic acids of white and red wines can be quantified with the following sample treatment: add 2 mL of 2 M NaOH to 20 mL of wine and heat for 15 min under reflux and with stirring. After cooling, adjust the pH of the solution to 10.0 with 1 M H_2SO_4 and complete the volume to 100 mL with distilled water. After this sample treatment, analyse an aliquot according to the general procedure, using a dilution of 1:5 and a sample volume of 0.1 mL.

The concentration obtained is the sum of the free and esterified D-/L-lactic acids [F + E] and, thus, the esterified D-/L-lactic acids concentration alone [E] can be determined as follows: [E] (g/l) = [F + E] – [F]

Determination of D- and L-lactic acids in beer

The D-/L-lactic acids concentration of beer can, usually, be determined without any sample treatment, except removal of carbon dioxide by stirring for approx. 1 min with a glass rod. In general, no dilution is necessary and a sample volume of 0.2 mL is appropriate.

Determination of D- and L-lactic acids in vinegar and vinegar-containing liquids

The D-/L-lactic acids concentration of vinegar or derived liquids can, usually, be quantified without any sample treatment, except filtration. Usually, no dilution is required and a sample volume of 0.1 mL is appropriate.

Determination of D- and L-lactic acids in yogurt and milk

Accurately weigh approx. 1 g of yogurt, or 10 g of milk, into a 100 mL volumetric flask containing 60 mL of distilled water. Add the following solutions and mix after each addition: 2 mL of Carrez I solution (3.60 g of potassium hexacyanoferrate (II) $\{K_4[Fe(CN)_6].3H_2O\}$ in 100 mL of distilled water), 2 mL of Carrez II solution (7.20 g of zinc sulphate ($ZnSO_4.7H_2O$) in 100 mL of distilled water) and 4 mL of NaOH solution (100 mM). Adjust volume to 100 mL with distilled water, mix and filter. Usually, no further dilution is required and sample

volumes of 0.1 mL (for yogurt) and 1.0 mL (for milk) are appropriate.

Determination of D- and L-lactic acids in cheese

Accurately weigh approx. 1 g of cheese into a 100 mL volumetric flask containing approx. 70 mL of distilled water and heat at 60 °C shaking several times for 20 min, or until fully dispersed. Adjust volume to 100 mL with distilled water, store at 0 to 4 °C for approx. 20 min to allow separation of the fat, and then filter. Usually, no dilution is required and a sample volume of 0.1 mL is appropriate.

Determination of D- and L-lactic acids in meat products

Accurately weigh approx. 5 g of homogenised sample into a beaker containing 20 mL of 1 M perchloric acid and homogenise using a disperser for 5 min. Add approx. 40 mL of distilled water and adjust the pH to approx. 10.0 with 2 M KOH. Transfer the contents to a 100 mL volumetric flask and fill to the mark with distilled water (if a fat layer develops, make sure this is above the mark, and the aqueous layer is at the mark). Store at 0 to 4 °C for approx. 20 min to allow separation of fat and precipitation of potassium perchlorate. Filter, discarding the first few mL of filtrate. For the assay, use the clear possibly slightly turbid solution diluted, if necessary. Usually, a dilution of 1:2 and a sample volume of 0.1 mL are appropriate.

References

Noll, F. (1988). L-(+)-Lactate. In Methods of Enzymatic Analysis (Bergmeyer, H. U., ed.), 3rd ed.,Vol.VI, pp. 582-588,VCH Publishers (UK) Ltd., Cambridge, UK.

Gawehn, K. (1988). D-(-)-Lactate. In Methods of Enzymatic Analysis (Bergmeyer, H. U., ed.), 3rd ed., Vol VI, pp. 588-592,VCH Publishers (UK) Ltd., Cambridge, UK.

Recommendations

The stereo-specific enzymatic determination of D-lactic (AK00121) and L-lactic acid (AK00131) are recommended/approved by the:
- European, German, International and Russian standards (EN, DIN, ISO, GOST);
- Contained in European Commission Regulation (analysis of wine);
- Recommended by the International Wine Office (OIV), the International Dairy Federation (IDF) and the International Federation of Fruit Juice Producers (IFU), by the Association of the Industry of Juices and Nectars from Fruits and Vegetables of the European Economic Community (A.I.J.N.)

Released 12/12

Certificate of Analysis

Test	Criteria	Result
Test Performance	Reaction completed within time stated	Meets specification
	Target value for recommended standard material +/- 10%	Meets specification
Blank reaction absorbance	+/- 10% of the blank value	Meets specification

Approved by:

José Prates
Senior Manager, Quality Systems

Please enquire info@nzytech.com to obtain any additional information about this kit, including additional specific applications.

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Estrada do Paço do Lumiar,
Campus do Lumiar - Edifício E, R/C
1649-038 Lisboa, Portugal
Tel.:+351.213643514
Fax: +351.217151168
www.nzytech.com