L-Lactic acid, UV method

Catalogue number: AK00131, 50 tests

Application

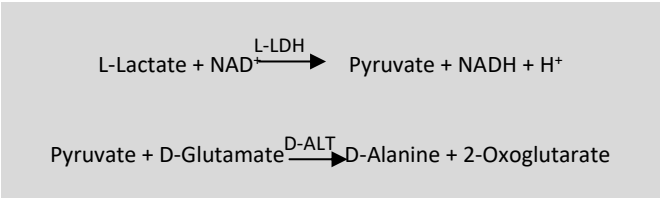
This rapid and simple stereo-specific enzymatic method is used for the determination of L-(+)-lactic acid (L-(+)-lactate) in foodstuffs such as milk and milk products (e. g. cheese, yogurt), wine, beer, bread and bakery goods, baking agents and sourdough, dietetic food, fruit and vegetable products (e. g. juices, jam, tomato pulp), meat products, soft drinks and lemonades, vinegar, as well as in animal feed, cosmetics, paper and cardboard, pharmaceuticals and biological samples. See also our test kit for D-/L-lactic acid (cat. n. AK00141).

Introduction

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 content of L-lactate in beer indicates the presence of *Lactobacilli* in production. 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 content of L-lactate in liquid whole egg or in egg powder gives good information about the hygienic situation of the products. Commercial lactic acid may not contain the stereo-isomeric forms in the ratio 1:1. Free L-lactic acid in the presence of water/moisture tends to form the dimer lactyl-lactate which does not react in the enzymatic determination; therefore, this material cannot be used for the production of standard solutions.

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:



The amount of NADH formed through the combined action of L-lactate dehydrogenase (L-LDH; EC 1.1.2.3) and D-alanine aminotransferase / D-glutamic-pyruvate transaminase (ALT/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).

Specificity

The determination is specific for L-lactic acid.

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 an L-lactic acid concentration of 0.107 mg/L sample solution when measured at 340 nm. 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 0.3 to 30 µg L-lactic acid per assay (v = 1.50 mL). 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 L-lactic acid, v = 1.50 mL). The CV is approx. 1 to 3% in the measuring range.

Kit composition

Solution 1. 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. Store at 2°C to 8 °C.

Solution 2. NAD^+ (380 mg) and PVP (60 mg). Store at 2°C to 8 °C. (Long-term storage at -30°C to -15 °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. 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). Store at 2°C to 8 °C. Swirl before use.

Suspension 4L. L-Lactate dehydrogenase (L-LDH, 2000 U/mL) in 3.2 M ammonium sulphate (1.1 mL). Store at 2°C to 8 °C. Swirl before use.

Solution 5. L-Lactic acid standard solution (5 mL, 0.15 mg/mL). Store at 2°C to 8 °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 L-lactic acid are not hazardous materials (see Hazardous Substances Regulations). However, the general safety measures that apply to all chemical substances should be followed.

Procedure (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 L-lactic acid (g/L), based on the ⍰ of NADH at 340 nm (6300 $L \times mol^{-1} \times cm^{-1}$), is calculated as follows:

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

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 L-lactic acid has been completed within the time specified in the assay (approx. 10 min), no interference has occurred. However, this can be further checked by adding 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.

Small amounts of GlDH in AST/GOT may cause reagent-dependent creep reactions, which may be eliminated by extrapolation or, better, by measuring the absorbance of blank and sample immediately one after the other.

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

General information on sample preparation

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

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 L-lactic acid in white and red wines

The free L-lactic acid 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.

To quantify the concentration of both free and esterified L-lactic acid [F + E] in white and red wines proceed as follows: add 2 mL of 2 M NaOH to 20 mL of wine and heat under reflux for 15 min with stirring. After cooling, adjust the pH of the solution to 10.0 with 1 M H_2SO_4 and adjust the volume to 100 mL with distilled water. Then analyse the sample according to the general procedure, with dilution if necessary. Typically, no further dilution is required and a sample volume of 0.1 mL is satisfactory.

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

Determination of L-lactic acid in beer

The L-lactic acid concentration of beer can, generally, be determined without any sample treatment, except removal of carbon dioxide by stirring for approx. 1 min with a glass rod.

Typically, no dilution is required and a sample volume of 0.2 mL is appropriate.

Determination of L-lactic acid in vinegar and vinegar-containing liquids

The L-lactic acid concentration of vinegar or other preserving liquids can usually be quantified without any sample treatment, except filtration and dilution where necessary). Typically, no dilution is required and a sample volume of 0.1 mL is appropriate.

Determination of L-lactic acid in yogurt and milk

Accurately weigh approx. 1 g of homogenised 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. Typically, no further dilution is required and sample volumes of 0.1 mL (for yogurt) and 1.0 mL (for milk) are appropriate.

Determination of L-lactic acid in cheese

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

Determination of L-lactic acid 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 4ºC 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. Typically, a dilution of 1:2 and 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 L- and of D-lactic (AK00121) acids 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.)

V2201

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:

Patrícia Ponte
Senior Manager, Quality Systems

For research use only

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

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