L-Lactic acid, UV method

Alternative Procedures

Micro-volumes formats

This kit has been developed to work in cuvettes with a standard pathlength of 1 cm, as described in the respective "Product Brochure". However, it can be adapted for use in 96-well microplates or in auto-analysers (micro-volume formats) with minimal assay optimisation. 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 the standard cuvette pathlength of 1 cm. Thus, to perform the calculation of the amount of analyte in the samples under analysis follow one of the three strategies described in the section below.

Auto-analyser procedure

This procedure can be performed using either a single point or a full calibration curve. For each batch of samples, either a single point standard or a calibration curve must be performed using the same batch of reagents.

Reagent preparation is accomplished as follows:

Preparation of R1:

Component	Volume
Solution 1	5.0 mL
Solution 2 (after addition of 5.5 mL of H_2O)	1.0 mL
Suspension 3 (swirl before use)	0.2 mL
Distilled water	12.9 mL
Total	19.1 mL

Preparation of R2:

Component	Volume
Suspension 4 (swirl before use)	0.2 mL
Distilled water	2.2 mL
Total	2.4 mL

Example Procedure:

	Volume
R1	0.200 mL
Sample	0.002 mL*
R2	0.025 mL

Reaction time: ~10 min at at 37 °C

Wavelength: 340 nm

Prepared reagent stability: > 2 days when refrigerated

Calculation: endpoint

Reaction direction: increase

Linearity: up to 310 µg/mL of L-Lactic acid in final reaction mixture

∗ If AU values are higher than 2, please dilute the sample with distilled water accordingly.

Strategies for analyte calculation

Auto-analysers use reaction volumes of approximately 0.315 ml and pathlengths from 4 to 8 mm, which is similar to a standard 96-well microplate in which the same reaction volume would have a pathlength of 6 or 7 mm (similar assay volumes). Therefore, in both formats (96-well microplate and auto-analysers systems), the calculation of the analyte must be done by one of the three possible methods described below:

1. Using the pathlength conversion factor

This is the easiest method to perform the calculation of the analyte. However, it requires a microplate reader with pathlength conversion capacity, i.e., the apparatus can detect the pathlength of each well and convert the individual readings to a 1 cm pathlength (cuvette format). In the case of auto-analysers, the absorbance readings should be directly converted to a 1 cm pathlength. This will allow the calculation of the analyte content as described in the "Product Brochure", provided with the kit and available at the NZYTech website.

2. Using one standard curve

In this method, it is necessary to perform a standard curve of the analyte on each microplate that contains the test samples, or in the auto-analyser, and calculate the result from the standard curve of analyte concentration vs. absorbance. The standard curve can be performed by using the control solution provided in the kit.

3. Using two standard curves

The most complicated method is to perform standard curves of the analyte in both the cuvette format (i.e. with a 1 cm of radiation pathlength) and the 96-well microplate or auto-analyser formats, and use these results to obtain a mean conversion factor between the cuvette procedure values and the alternative procedure values.

The standard curves can be performed by using the control solution provided in the kit.

V1901

nzytech
genes & enzymes

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

AK0013_IFU_EN_V2302

L-Lactic acid, UV method

Catalogue number	Presentation
AK00131	50 tests

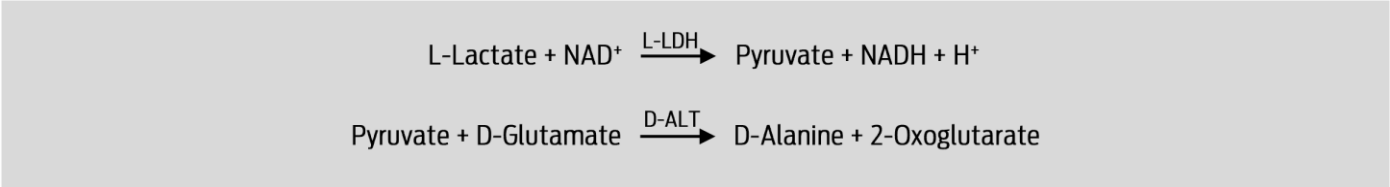
Application

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



The determination of L-lactic acid requires 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 -30°C to -15 °C between use and keep cool during use. Once dissolved, the reagent is stable until expiry date, at -30 °C to -15 °C.

Suspension 3. D-Alanine aminotransferase (D-ALT) 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) in 3.2 M ammonium sulphate (1.1 mL). Store at 2 °C to 8 °C. Swirl before use.

Solution 5L. 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×mol^{-1}×cm^{-1}).

Protocol (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 co

Calculation

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

$$C\ (\text{L-Lactic acid}) = 0.3204 \times \Delta A_{\text{L-Lactic acid}} \quad [g/L]$$

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”, 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 °C to 4 °C 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.

Recommendations

The stereo-specific enzymatic determination of L- and of D-lactic 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.)

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