L-Lactic acid

Enzymatic BioAnalysis
Food Analysis

UV method

for the determination of L-lactic acid in foodstuffs and other materials

Determination of D-lactic acid, see under Pt. 11.

Not for use in diagnostic procedures for clinical purposes
FOR *IN VITRO* USE ONLY

Cat. No. 139 084
Test-Combination for approx. 25 determinations

For recommendations for methods and standardized procedures see references (2)

Principle (Ref. 1)

L-Lactic acid (L-lactate) is oxidized by nicotinamide-adenine dinucleotide (NAD) in the presence of L-lactate dehydrogenase (L-LDH) to pyruvate (1).

(1) L-Lactate + NAD^+ $\overset{\text{L-LDH}}{\rightleftharpoons}$ pyruvate + NADH + H^+

The equilibrium of this reaction lies almost completely on the side of L-lactate. However, by trapping pyruvate in a subsequent reaction catalyzed by the enzyme glutamate-pyruvate transaminase (GPT) in the presence of L-glutamate, the equilibrium can be displaced in favour of pyruvate and NADH (2).

(2) Pyruvate + L-glutamate $\overset{\text{GPT}}{\rightleftharpoons}$ L-alanine + 2-oxoglutarate

The amount of NADH formed in the above reaction is stoichiometric to the amount of L-lactic acid. The increase in NADH is determined by means of its light absorbance at 334, 340 or 365 nm.

The Test-Combination contains:

1. Bottle 1 with approx. 30 ml solution, consisting of: glycylglycine buffer, pH 10.0; L-glutamic acid, 440 mg; stabilizers
2. Bottle 2 with approx. 210 mg NAD, lyophilisate
3. Bottle 3 with approx. 0.7 ml glutamate-pyruvate transaminase suspension, approx. 1100 U
4. Bottle 4 with 0.7 ml L-lactate dehydrogenase solution, approx. 3800 U
5. L-Lactate standard solution for assay control purposes (measurement of the standard solution is not necessary for calculating the results.)

Preparation of solutions

1. Use contents of bottles 1, 3 and 4 undiluted.
2. Dissolve contents of bottle 2 with 6 ml redist. water.

Stability of reagents

The contents of bottles 1, 2, 3 and 4 are stable for 1 year at +4°C. Bring solution 1 to 20–25°C before use.
Solution 2 is stable for 3 weeks at +4°C, and for 2 months at –20°C[1].

Procedure

Wavelength[2]: 340 nm, Hg 365 nm or Hg 334 nm
Glass cuvette[3]: 1.00 cm light path
Temperature: 20–25°C
Final volume: 2.240 ml
Read against air (without a cuvette in the light path), against water or against blank[4]
Sample solution: 0.3–35 µg of L-lactic acid/cuvette[5] (in 0.100–1.000 ml sample volume)

Pipette into cuvettes	Blank	Sample
solution 1	1.000 ml	1.000 ml
solution 2	0.200 ml	0.200 ml
suspension 3	0.020 ml	0.020 ml
sample solution*	–	0.100 ml
redist. water	1.000 ml	0.900 ml
Mix**, read absorbances of the solutions (A_1) after approx. 5 min. Start reaction by addition of:		
solution 4	0.020 ml	0.020 ml
Mix**, after completion of the reaction (approx. 20 min) read absorbances of blank and sample (A_2) immediately one after another (see pt. 2.4).		

* Rinse the enzyme pipette or the pipette tip of the piston pipette with sample solution before dispensing the sample solution.

** For example, with a plastic spatula or by gentle swirling after closing the cuvette with Parafilm® (registered trademark of the American Can Company, Greenwich, Ct., USA)

Determine the absorbance differences (A_2–A_1) for both blank and sample. Subtract the absorbance difference of the blank from the absorbance difference of the sample.

$$\Delta A = (A_2–A_1)_{sample} – (A_2–A_1)_{blank}$$

The measured absorbance differences should, as a rule, be at least 0.100 absorbance units to achieve sufficiently accurate results (see "Instructions for performance of assay").

Calculation

According to the general equation for calculating the concentration:

$$c = \frac{V \times MW}{\varepsilon \times d \times v \times 1000} \times \Delta A \text{ [g/l]}$$

V = final volume [ml]
v = sample volume [ml]
MW = molecular weight of the substance to be assayed [g/mol]
d = light path [cm]
ε = extinction coefficient of NADH at
340 nm = 6.3 [l x $mmol^{-1}$ x cm^{-1}]
Hg 365 nm = 3.4 [l x $mmol^{-1}$ x cm^{-1}]
Hg 334 nm = 6.18 [l x $mmol^{-1}$ x cm^{-1}]

It follows for L-lactic acid:

$$c = \frac{2.240 \times 90.1}{\varepsilon \times 1.00 \times 0.100 \times 1000} \times \Delta A = \frac{2.018}{\varepsilon} \times \Delta A \text{ [g L-lactic acid/l sample solution]}$$

If the sample has been diluted during preparation, the result must be multiplied by the dilution factor F.

When analyzing solid and semi-solid samples which are weighed out for sample preparation, the result is to be calculated from the amount weighed:

$$\text{Content}_{\text{L-lactic acid}} = \frac{c_{\text{L-lactic acid}} \text{ [g/l sample solution]}}{\text{weight}_{\text{sample}} \text{ in g/l sample solution}} \times 100 \text{ [g/100 g]}$$

1. Instructions for performance of assay

The amount of L-lactic acid present in the cuvette has to be between 0.5 µg and 35µg (measurement at 365 nm) or 0.3 µg and 20 µg (measurement at 340, 334 nm), respectively. In order to get a sufficient absorbance difference, the sample solution is diluted to yield a L-lactic acid concentration between 0.06 and 0.35 g/l or 0.03 and 0.2 g/l, respectively.

Dilution table

Estimated amount of L-lactic acid per liter measurements at 340 or 334 nm	365 nm	Dilution with water	Dilution factor F
< 0.2 g	< 0.35 g	–	1
0.2–2.0 g	0.35–3.5 g	1 + 9	10
2.0–20 g	3.5 –35 g	1 + 99	100
> 20 g	> 35 g	1 + 999	1000

1 If desired, use NAD from Boehringer Mannheim GmbH, Biochemicals, Cat. No. 127 981: 35 mg NAD/ml, in redist. water.
2 The absorption maximum of NADH is at 340 nm. On spectrophotometers, measurements are taken at the absorption maximum; if spectralline photometers equipped with a mercury vapor lamp are used, measurements are taken at a wavelength of 365 nm or 334 nm.
3 If desired, disposable cuvettes may be used instead of glass cuvettes.
4 For example, when using a double-beam photometer
5 See instructions for performance of the assay
6 Available from Boehringer Mannheim GmbH, Biochemicals

If the measured absorbance difference (ΔA) is too low (e. g., < 0.100), the sample solution should be prepared again (weigh out more sample or dilute less strongly) *or* the sample volume to be pipetted into the cuvette can be increased up to 1.000 ml. The volume of water added must then be reduced so as to obtain the same final volume in the cuvettes for the sample and blank. The new sample volume v must be taken into account in the calculation.

2. Technical information

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

2.2 In carrying out the calculation, a clear indication should be given as to whether the results are to be given as L-lactic acid (molar mass 90.1 g/mol) or as L-lactate (molar mass 89.1 g/ mol). (In enzymatic determinations, the L-lactate ion is measured.)

2.3 In evaluating the analytical results, it should be taken into account that in the acidimetric determination of 'total acid calculated as lactic acid' protons are measured and in the enzymatic determination the L-lactate ion is measured. It is thus not possible to compare such results directly.

2.4 There may be a reagent-dependent creep reaction at A_2 after the quantitative conversion of L-lactic acid. An extrapolation of the absorbances back to the time of the addition of solution 4 (L-LDH) is not necessary when the absorbances of blank and sample are read immediately one ofter the other. If there is an additional sample dependent creep reaction, the correct A_2 value has to be determined by extrapolation of the absorbances back to the addition of solution 4 (L-LDH).

2.5 Commercial lactic acid may not contain the stereo-isometric forms in the ratio 1 : 1. Furthermore, free lactic acid forms partially the dimer lactyl-lactate which does not react in the enzymatic determination. Therefore, free lactic acid should not be used for the preparation of standard solutions. The lithium and also the calcium salts are suited for the use as standards.

In the analysis of commercial lithium-L-lactate (molecular weight 96.0), results of approx. 98% have to be expected.

3. Specificity (Ref. 1)

The method is specific for L-lactic acid.

4. Sensitivity and detection limit (Ref. 1)

The smallest differentiating absorbance for the procedure is 0.005 absorbance units. This corresponds to a maximum sample volume v = 1.000 ml and measurement at 340 of a L-lactic acid concentration of 0.15 mg/l sample solution (if v = 0.100 ml, this corresponds to 1.5 mg/l sample solution).

The detection limt of 0.3 mg/l is derived from the absorbance difference of 0.010 (as measured at 340 nm) and a maximum sample volume v = 1.000 ml.

5. Linearity

Linearity of the determination exists from 0.3 µg L-lactic acid/assay (0.3 mg L-lactic acid/l sample solution; sample volume v = 1.000 ml) to 35 µg L-lactic acid/assay (0.35 g L-lactic acid/l sample solution; sample volume v = 0.100 ml).

6. Precision

In a double determination using one sample solution, a difference of 0.005 to 0.010 absorbance units may occur. With a sample volume of v = 0.100 ml and measurement at 340 nm, this corresponds to a L-lactic acid concentration of approx. 1.5–3 mg/l. (If the sample is diluted during sample preparation, the result has to be multiplied by the dilution factor F. If the sample is weighed in for sample preparation, e.g. using 1 g sample/100 ml = 10 g/l, a difference of 0.015–0.03 g/100 g can be expected.)

The following data have been published in the literature:

L-Lactic acid:			
CV = 2.3%	lithium-L-lactate solution		(Ref. 1.3)
Yoghurt:	r = 0.05 g/100 g	R = 0.07 g/100 g	
Milk powder:	r = 0.008 g/100 g	R = 0.015 g/100 g	
Whole egg powder:			
x = 240 mg/kg	r = 62.8 mg/kg	$s_{(r)}$ = ± 22.2 mg/kg	
	R = 89.1 mg/kg	$s_{(R)}$ = ± 31.5 mg/kg	
For further data see references			(Ref. 2.1)
Wine:	$r = 0.02 + 0.07 \cdot x_i$	$R = 0.05 + 0.125 \cdot x_i$	
	x_i = L-lactic acid concentration in g/l		(Ref. 2.14, 2.15)
D-Lactic acid (s. pt. 11):			
CV = 1.96%	culture medium		(Ref. 1.5)
Yoghurt:	r = 0.03 g/100 g	R = 0.05 g/100 g	
Milk powder:	r = 0.0008 g/100 g	R = 0.003 g/100 g	
For further data see references			(Ref. 2.1)

7. Recognizing interference during the assay procedure

7.1 If the conversion of L-lactic acid has been completed according to the time given under 'determination', it can be concluded in general that no interference has occurred.

7.2 On completion of the reaction, the determination can be restarted by adding L-lactic acid or L-lactate (qualitative or quantitative): if the absorbance is altered subsequent to the addition of the standard material, this is also an indication that no interference has occurred.

7.3 Operator error or interference of the determination through the presence of substances contained in the sample can be recognized by carrying out a double determination using two different sample volumes (e.g. 0.100 ml and 0.200 ml): the measured differences in absorbance should be proportional to the sample volumes used.

When analyzing solid samples, it is recommended that different quantities (e.g. 1 g and 2 g) be weighed into 100 ml volumetric flasks. The absorbance differences measured and the weights of sample used should be proportional for identical sample volumes.

7.4 Possible interference caused by substances contained in the sample can be recognized by using an internal standard as a control: in addition to the sample, blank and standard determinations, a further determination should be carried out with sample **and** standard solution in the same assay. The recovery can then be calculated from the absorbance differences measured.

7.5 Possible losses during the determination can be recognized by carrying out recovery tests: the sample should be prepared and analyzed with and without standard material. The additive should be recovered quantitatively within the error range of the method.

8. Reagent hazard

The reagents used in the determination of L-lactic acid are not hazardous materials in the sense of the Hazardous Substances Regulations, the Chemicals Law or EC Regulation 67/548/EEC and subsequent alteration, supplementation and adaptation guidelines. However, the general safety measures that apply to all chemical substances should be adhered to.

After use, the reagents can be disposed of with laboratory waste, but local regulations must always be observed. Packaging material can be disposed of in waste destined for recycling.

9. General information on sample preparation

In carrying out the assay:
Use **clear, colorless and practically neutral liquid samples** directly, or after dilution according to the dilution table, and of a volume up to 1.000 ml;
Filter **turbid solutions**;
Degas **samples containing carbon dioxide** (e.g. by filtration);
Adjust **acid samples** to approx. pH 8–10 by adding sodium or potassium hydroxide solution;
Adjust **acid and weakly colored samples** to approx. pH 8–10 by adding sodium or potassium hydroxide solution and incubate for approx. 15 min;
Measure **'colored' samples** (if necessary adjusted to pH 8–9) against a sample blank (= buffer or redist. water + sample), adjust the photometer to 0.000 with the blank in the beam, especially if there is a creep reaction before the addition of solution 4 (L-LDH);
Treat **'strongly colored' samples** that are used undiluted or with a higher sample volume with polyvinylpolypyrrolidone (PVPP) or with polyamide, e.g. 1 g/100 ml;
Crush or homogenize **solid or semi-solid samples,** extract with water or dissolve in water and filter if necessary; resp. remove turbidities or dyestuffs by Carrez clarification;
Deproteinze **samples containing protein** with perchloric acid; Alternatively clarify with Carrez reagents;
Extract **samples containing fat** with hot water (extraction temperature should be above the melting point of the fat involved). Cool to allow the fat to separate, make up to the mark, place the volumetric flask in an ice bath for 15 min and filter; alternatively clarify with Carrez-solutions after the extraction with hot water.

Carrez clarification:

Pipette or weigh sufficient quantity of the sample into a 100 ml volumetric flask and add approx. 60 ml redist. water. Subsequently, carefully add 5 ml Carrez-I-solution (potassium hexacyanoferrate(II) (ferrocyanide), 85 mmol/l = 3.60 g $K_4[Fe(CN)_6 \times 3\ H_2O$/100 ml) and 5 ml Carrez-II-solution (zinc sulfate, 250 mmol/l = 7.20 g $ZnSO_4 \times 7\ H_2O$/100 ml). Adjust to pH 7.5–8.5 with sodium hydroxide (0.1 mol/l; e.g. 10 ml). Mix rigorously after each addition. Fill the volumetric flask to the mark, mix and filter.

Preparation of egg and egg product samples is dealt with in pt. 10 (application examples). Note: Treatment with concentrated Carrez-solutions has proved beneficial in routine analysis. In Germany, the

method has been standardized and published in § 35 of the Foodstuffs and Consumer Goods Law (Lebensmittel- and Bedarfsgegenständegesetz, LMBG). The sample solution resulting from Carrez clarification can also be used for the determination of D-3-hydroxybutyric acid and of succinic acid.

10. Application examples

Determination of L-lactic acid in fruit and vegetable juices and similar beverages
Filter turbid juices and dilute until a L-lactic acid concentration of approx. 0.03 to 0.35 g/l is obtained. The diluted solution can be used for the assay even if it is slightly colored.

Only strongly colored juices are to be decolorized if they are used undiluted for the assay. In such cases, proceed as follows:
Mix 10 ml of juice and approx. 0.1 g of polyamide powder or polyvinylpolypyrrolidone (PVPP), stir for 1 min, and filter. Use the clear, slightly colored solution for the assay.

Furthermore it is possible to use the Carrez clarification for sample preparation:
Accurately weigh approx. 1 to 10 g of sample into a 100 ml volumetric flask, or pipette 1 to 10 ml sample, add approx. 60 ml water; shake from time to time. For protein precipitation, add 5 ml Carrez-I-solution (3.60 g potassium hexacyanoferrate-II, $K_4[Fe(CN)_6]$ x 3 H_2O/100 ml), 5 ml Carrez-II-solution (7.20 g zinc sulfate, $ZnSO_4$ x 7 H_2O/100 ml) and 10 ml NaOH (0.1 mol/l), shake vigorously after each addition, adjust to room temperature and fill up with water to the mark, filter. Use the clear, possibly slightly turbid solution for the assay, diluted if necessary.
If esterified L-lactic acid is to be determined in addition to free L-lactic acid the esters must be converted to the free acid by hydrolysis. Proceed as stated under "wine".

Determination of free L-lactic acid in wine
Free L-lactic acid in white or red wine can usually be determined without prior dilution or decolorization.

Determination of free and esterified L-lactic acid in wine
In order to determine the total L-lactic acid content, heat 20 ml of wine and 2 ml sodium hydroxide (2 mol/l) for 15 min under a reflux condenser, allow to cool to room temperature and neutralize with sulfuric acid (1 mol/l; use indicator paper). Transfer solution quantitatively into a 50 ml volumetric flask and fill up to the mark with water. Use the sample for the assay according to the standard procedure.

Wines with a high sugar content are to be heated with water instead of NaOH under a reflux condenser for 15 min.

Determination of L-lactic acid in beer
For removal of the carbonic acid, stir approx. 5–10 ml of beer with a glass rod for approx. 1 min or filter. The largely CO_2-free sample may be used directly for the assay.

Determination of L-lactic acid in vinegar-containing liquids
Use 0.100 ml of wine vinegar or pickled cucumber juice directly for the assay. The high acetic acid content inhibits the assay. Therefore, wait for the end of the reaction (30–40 min) before reading absorbances A_2.

Determination of L-lactic acid in sauerkraut juice
Transfer 0.500 ml of sauerkraut juice into a 50 ml volumetric flask dilute to the mark with water and mix. Use the diluted solution (1 + 99) for the assay.

Determination of L-lactic acid in yoghurt (milk)
Mix 1 g of yoghurt (milk) with 49 ml water (using an electric mixer or homogenizer), filter, and use 0.100 ml of the filtrate for the assay.

Determination of L-lactic acid in cheese
Grind approx. 10 g of cheese and mix. Accurately weigh approx. 1 g of the sample into a 100 ml volumetric flask, add approx. 80 ml water, and heat for 15 min at approx. 60°C with occasional shaking. After cooling to room temperature fill up to 100 ml with water. To obtain separation of fat, keep the volumetric flask in the cold (refrigerator, ice-bath) for 15 min, filter solution. Use 0.100 ml (hard cheese) or 0.500 ml (soft cheese) of the clear filtrate for the assay.

Determination of L-lactic acid in meat products
Accurately weigh approx. 5 g of homogenized sample into a homogenizer beaker, add approx. 20 ml perchloric acid (1 mol/l) and homogenize for 10 min. Transfer the contents quantitatively into a beaker with approx. 40 ml water. Adjust to pH 10–11 with potassium hydroxide (2 mol/l) while stirring (magnetic stirrer). Transfer contents quantitatively into a 100 ml volumetric flask with water, fill up to the mark with water, whereby it must be taken care that the fatty layer is above the mark and the aqueous layer is **at** the mark. Shake the mixture. For separation of fat and for precipitation of the potassium perchlorate refrigerate for 20 min and filter. The first few ml are to be discarded. Use the clear, possibly slightly turbid solution diluted, if necessary, for the assay.

Determination of L-lactic acid in liquid whole egg (Ref. 2.1)
Accurately weigh approx. 5 g homogenized whole egg into a 25 ml-volumetric flask, add 10 ml redist. water, one drop n-octanol, mix and heat for 15 min in a water-bath (approx. 100°C). Allow to cool to room temperature, and add one after the other and shake after each addition: 1 ml concentrated Carrez-I-solution (15.0 g potassium hexacyanoferrate-II, $K_4Fe(CN)_6$] x 3 H_2O/100 ml), 1 ml concentrated Carrez-II-solution (30.0 g zinc sulfate, $ZnSO_4$ x 7 H_2O/100 ml). Fill up to the mark with NaOH (0.1 mol/l), mix and filter. Use filtrate for the assay (v = 0.100 ml, when using microbial contaminated egg; v = 0.500 ml, when using fresh egg). The altered sample volume must be taken into account in the calculation.

Determination of L-lactic acid in whole egg powder (Ref. 2.1)
Accurately weigh approx. 1 g whole egg powder into a 25 ml volumetric flask, add 12 ml redist water and one drop n-octanol, mix and heat for 15 min in a water-bath (approx. 100°C). Allow to cool to room temperature, add one after the other and shake vigorously after each addition: 1 ml concentrated Carrez-II-solution (15.0 g potassium hexacyanoferrate-II, $K_4[Fe(CN)_6]$ x 3 H_2O/100 ml), 1 ml concentrated Carrez-II-solution (30.0 g zinc sulfate, $ZnSO_4$ x 7 H_2O/100 ml). Adjust to pH 8–9 with NaOH (1 mol/l), fill up to the mark with redist. water, mix and filter. Use 0.100–0.500 ml filtrate for the assay. The altered sample volume must be taken into account in the calculation.

11. Determination of D-lactic acid (Ref. 1.5, 1.6)

Following the instructions outlined above, it is also possible to determine D-lactic acid when solution 4 is replaced by the enzyme D(-)-lactate dehydrogenase.

(The determination can also be carried out by using Test-Combination D-Lactic acid/L-Lactic acid, Cat. No. 1112821[6].)

Enzyme
D(-)-lactate dehydrogenase (D-LDH) from *Lactobacillus leichmannii*, Cat. No. 106941[6].
Pack size 5 mg/ml, for 20 determinations

Experimental conditions
The experimental conditions conform to those stated for the assay of L-lactic acid.
Required volume of enzyme suspension: 0.050 ml

Reaction time: approx. 30 min; to shorten the reaction time, measurements may also be carried out at 37°C (reaction time: approx. 20 min).

Procedure

Pipette into cuvettes	Blank	Sample
solution 1	1.000 ml	1.000 ml
solution 2	0.200 ml	0.200 ml
suspension 3	0.020 ml	0.020 ml
sample solution	–	0.100 ml
redist. water	1.000 ml	0.900 ml
Mix and read absorbances of the solutions (A_1) after approx. 5 min. Start reaction by addition of:		
D(-)-LDH	0.050 ml	0.050 ml
Mix, after completion of the reaction (approx. 30 min) read absorbances of the solutions (A_2) immediately one after another.		

Determine the absorbance differences (A_2–A_1) for both blank and sample. Subtract the absorbance difference of the blank from the absorbance difference of the sample.

$$\Delta A = (A_2 - A_1)_{sample} - (A_2 - A_1)_{blank}$$

Calculation

$$c = \frac{2.270 \times 90.1}{\varepsilon \times 1.00 \times 0.100 \times 1000} \times \Delta A = \frac{2.045}{\varepsilon} \times \Delta A$$

[g D-lactic acid/l sample solution]

D-lactic acid can be determined directly after the determination of the L-lactic acid in the same cuvette. In this case, 0.050 ml D-LDH are to be added to blank and sample after the L-lactic acid reaction has come to completion. The altered final volume must be taken into account in the calculation formula.

Specificity
This method is specific for D-lactic acid.

In the analysis of commercial lithium-D-lactate (molecular weight 96.0), results of approx. 99% have to be expected.

12. Determination of lactic acid esters
(e.g. glyceride lactic acid esters, emulsifiers)

In monoglyceride or diglyceride lactic acid esters bound lactic acid can also be determined simultaneously with free lactic acid (lactate), by extracting the sample with chloroform and saponifying of the esters subsequently with potassium hydroxide solution. Proceed as follows:

Boil the pulverized and homogenized sample which contains up to 200 mg monoglyceride lactic acid ester (e.g. monooleyl-L-lactyl-glyceride ester, MW approx. 445) or up to 250 mg diglyceride lactic acid ester (e.g. dioleyl-L-lactyl-glyceride ester, MW approx. 535) with approx. 50 ml chloroform for approx. 2 h in a 250 ml round-bottomed flask under a reflux condenser.

Filter and wash the precipitate with chloroform. Evaporate the chloroform in a rotation evaporator. Boil the residue, evaporated to nearly dryness, with 25 ml methanolic KOH (1 mol/l) for 10 min under a reflux condenser. Allow solution to cool to room temperature, and neutralize or acidify slightly, respectively, with approx. 5 ml HCl (5 mol/l). Transfer quantitatively into a 100 ml volumetric flask, fill up to the mark with water, mix and filter. Use the relatively clear solution for the assay.

Determine L- or D-lactic acid, respectively. For determination of the content the molecular weight of the glyceride has to be taken into account.

13. Further applications
The method may also be used in the examination of cosmetics (Ref. 5.1), paper, pharmaceuticals and in research when analyzing biological samples. For details of sampling, treatment and stability of the sample see Ref. 1.3, 1.4.

13.1 Determination of L-lactic acid in blood (Ref. 1.4)
Mix 2.000 ml of blood with 4.000 ml of ice-cold perchloric acid (0.6 mol/l) in a centrifuge tube. Place the mixture in an ice-bath for 10 min and centrifuge. Add 0.010 ml methyl orange solution (0.05%; w/v) to 4.000 ml of the supernatant and neutralize by addition of 0.170 ml potassium hydroxide (3 mol/l) (mixture becomes salmon-pink). Place the mixture again in an ice-bath for 15 min and filter. Use the filtrate for the assay.

The dilution factor F (depending on sample preparation) is obtained from the sample volume (2.000 ml), the volumes of perchloric acid (4.000 ml), the volume of supernatant (4.000 ml), of the methyl orange solution (0.010 ml), and of potassium hydroxide (0.170 ml), the specific gravity of the sample material (1.06 g/ml blood) and the fluid content (0.80 in the case of blood):

$$F_{blood} = \frac{(2.000 \times 1.06 \times 0.80 + 4.000) \times (4.000 + 0.010 + 0.170)}{2.000 \quad x \quad 4.000} = 2.98$$

Calculation:

$$c = \frac{2.018 \times \Delta A \times F}{\varepsilon}$$ [g L-lactic acid/l sample]

$$c = \frac{22.40 \times \Delta A \times F}{\varepsilon}$$ [mmol L-lactic acid/l sample]

Wavelength	Hg 365 nm	340 nm	Hg 334 nm
c [g/l]	1.769 x ΔA	0.9546 x ΔA	0.9731 x ΔA
c [mmol/l]	19.63 x ΔA	10.60 x ΔA	10.80 x ΔA

13.2 Determination of L-lactic acid in serum (Ref. 1.4)
Mix 3.000 ml of serum with 3.000 ml of ice-cold perchloric acid (0.6 mol/l) in a centrifuge tube. Place the mixture in an ice-bath for 10 min and centrifuge. Add 0.010 ml methyl orange solution (0.05%; w/v) to 4.000 ml of the supernatant and neutralize by addition of 0.170 ml potassium hydroxide (3 mol/l) (mixture becomes salmon-pink). Place the mixture again in an ice-bath for 15 min and filter. Use the filtrate for the assay.

The dilution factor F (depending on sample preparation) is obtained from the sample volume (3.000 ml), the volumes of perchloric acid (3.000 ml), of supernatant (4.000 ml), of the methyl orange solution (0.010 ml), and of potassium hydroxide (0.170 ml), the specific gravity of the sample material (1.03 g/ml serum) and the fluid content (0.92 in the case of serum):

$$F_{serum} = \frac{(3.000 \times 1.03 \times 0.92 + 3.000) \times (4.000 + 0.010 + 0.170)}{3.000 \quad x \quad 4.000} = 2.035$$

Calculation:

$$c = \frac{2.018 \times \Delta A \times F}{\varepsilon}$$ [g L-lactic acid/l sample]

$$c = \frac{22.40 \times \Delta A \times F}{\varepsilon}$$ [mmol L-lactic acid/l sample]

Wavelength	Hg 365 nm	340 nm	Hg 334 nm
c [g/l]	1.208 x ΔA	0.6519 x ΔA	0.6645 x ΔA
c [mmol/l]	13.41 x ΔA	7.236 x ΔA	7.376 x ΔA

13.3 Determination of L-lactic acid in urine and in cerebrospinal fluid
Use the sample for the assay, diluted according to the dilution table, if necessary (dilution factor = F).

Calculation:

$$c = \frac{2.018 \times \Delta A \times F}{\varepsilon}$$ [g L-lactic acid/l sample]

$$c = \frac{22.40 \times \Delta A \times F}{\varepsilon}$$ [mmol L-lactic acid/l sample]

Wavelength	Hg 365 nm	340 nm	Hg 334 nm
c [g/l]	0.5935 x ΔA x F	0.3203 x ΔA x F	0.3265 x ΔA x F
c [mmol/l]	6.588 x ΔA x F	3.556 x ΔA x F	3.625 x ΔA x F

13.4 Determination of L-lactic acid in fermentation samples and cell culture media
Place the sample (after centrifugation, if necessary) in a water-bath at 80°C for 15 min to stop enzymatic reactions. Centrifuge and use the supernatant (diluted according to the dilution table, if necessary) for the assay. Alternatively, deproteinization can be carried out with perchloric acid or with Carrez-solutions. See the above-mentioned examples. Homogenize gelatinous agar media with water and treat further as described.

References

1.1 Gutmann, I. & Wahlefeld, A.W. (1974) in Methoden der enzymatischen Analyse (Bergmeyer, H.U., Hrsg.) 3. Aufl., Bd. 2, 1510–1514; Verlag Chemie, Weinheim, and (1974) in Methods of Enzymatic Analysis (Bergmeyer, H.U., ed.) 2nd ed., vol. 3, pp. 1464–1468; Verlag Chemie, Weinheim/Academic Press, Inc., New York and London

1.2 Noll, F. (1966) Methode zur quantitativen Bestimmung von L(+)-Lactat mittels Lactat-Dehydrogenase und Glutamat-Pyruvat-Transaminase, Biochem.Z. **346**, 41–49

1.3 Noll, F. (1974) in Methoden der enzymatischen Analyse (Bergmeyer, H.U., Hrsg.) 3. Aufl., Bd. 2, S. 1521–1525; Verlag Chemie, Weinheim, and (1974) in Methods of Enzymatic Analysis (Bergmeyer, H.U., ed.) 2nd ed., vol. 3, pp. 1475–1479; Verlag Chemie, Weinheim/Academic Press, Inc., New York and London

1.4 Noll, F. (1984) in Methods of Enzymatic Analysis (Bergmeyer, H.U., ed.) 3rd ed., vol. VI, pp. 582–588, Verlag Chemie, Weinheim, Deerfield Beach/Florida, Basel

1.5 Gawehn, K. & Bergmeyer, H.U. (1974) in Methoden der enzymatischen Analyse (Bergmeyer, H.U., Hrsg.) 3. Aufl., Bd. 2, S. 1538–1541; Verlag Chemie, Weinheim, and (1974) in Methods of Enzymatic Analysis (Bergmeyer, H.U., ed.) 2nd ed., vol. 3, pp. 1492–1495; Verlag Chemie, Weinheim/Academic Press, Inc., New York and London

1.6 Gawehn, K. (1984) in Methods of Enzymatic Analysis (Bergmeyer, H.U., ed.) 3rd ed., vol. VI, pp. 588–592, Verlag Chemie, Weinheim, Deerfield Beach/Florida, Basel

2.1 Amtliche Sammlung von Untersuchungsverfahren nach §35 LMBG; Untersuchung von Lebensmitteln: Bestimmung von L- und D-Milchsäure (L- und D-Lactat) in Fleischerzeugnissen, 07.00-15 (November 1981); Bestimmung von L- und D-Milchsäure in Wurstwaren, 08.00-17 (November 1981); Bestimmung von L- und D-Milchsäure (L- und D-Lactat) in Milch und Milchprodukten, 01.00-26 (Juni 1987); Bestimmung von L- und D-Milchsäure (L- und D-Lactat) in Milchprodukten, 02.00-16 (Juni 1987); Bestimmung von L-Milchsäure, Bernsteinsäure und D-3-Hydroxybuttersäure in Ei und Eiprodukten, 05.00-2 (November 1987)

2.2 Schweizerisches Lebensmittelbuch, Kapitel 61B (Enzymatische Bestimmungen)/3.3. (1981), Kapitel 1 (Milch)/5.2 (1987), Kapitel 2B (Sauermilchprodukte)/18 (1980), Kapitel 28A (Frucht- und Gemüsesäfte u.a.)/7.5 (1988)

2.3 Gombocz, E., Hellwig, E., Vojir, F. & Petuely, F. (1981) Deutsche Lebensmittel-Rundschau **77**, 6–7

2.4 Brautechnische Analysenmethoden, Band III, S. 572–576 (1982), Methodensammlung der Mitteleuropäischen Brautechnische Analysenkommission (MEBAK), herausgegeben von F. Drawert im Selbstverlag der MEBAK, Freising

2.5 Intl. Federation of Fruit Juice Producers (IFU, Methods of Analysis, no. 53–1983)

2.6 International Standard (ISO) 8069: Dried milk – Determination of lactic acid and lactates content – Enzymatic method (Juni 1986)

2.7. Ministero dell' Agricoltura e delle Foreste (1986) Approvazione dei "Metodi ufficiali di analisi per i mosti, i vini, gli agri di vino (aceti) e i sottoprodotti della vinificazione". Gazzetta Ufficiale della Repubblica Italiana, n. 161 del 14 luglio 1986

2.8 Ministero dell' Agricoltura e delle Foreste (1986) Approv. dei "Metodi ufficiali di analisi per i formaggi". Gazzetta Ufficiale della Repubblica Italiana, n. 229 del 2 ottobre 1986

2.9 Niederlande: Warenwet, Uitvoeringsvoorschriften (C II-6), Regeling Onderzoekingsmethoden voor brood; Methode 17: De Bepaling van Melkzuur (Oktober 1986); Dit voorschrift betrift een methode voor de bepaling van melkzuur (lactaten) in het in brood verwerkte meel
2.10 International Dairy Federation, International IDF Standard 69B: 1987: Dried Milk, Determination of Lactic Acid and Lactates Content, Enzymatic Method
2.11 RSK-Values, The Complete Manual, Guide Values and Ranges of Specific Numbers for Fruit Juices and Nectars, Including the Revised Methods of Analysis (1987), 1st ed., Verlag Flüssiges Obst/Liquid Fruit, D-56370 Eschborn, pp. 107–111
2.12 Deutsche Norm, Bestimmung des Gehaltes an L- und D-Milchsäure (L- und D-Lactat) in Milch und Milchprodukten, Enzymatisches Verfahren, DIN 10 335 (Juni 1987).
2.13 Council Directive of 20 June 1989 on hygiene and health problems affecting the production and the placing on the market of egg products (89/437/EEC), Official Journal No. L 212, 22/07/89 p. 0087
2.14 Recueil des méthodes internationales d'analyse des vins et des moûts, Complément n° 1 à l'édition officielle de juin 1990, OFFICE INTERNATIONAL DE LA VIGNE ET DU VIN, S. 179–182
2.15 Official Journal of the European Communities L 272 (3 October 1990), Legislation: Commission Regulation (EEC) No 2676/90 of 17 September 1990 determining Community methods for the analysis of wines (pp. 97–100)
2.16 Verband Deutscher Landwirtschaftlicher Untersuchungs- und Forschungsanstalten, VDLUFA (1993) Methodenbuch Band VI, C 8.6
3.1 Duran, M., van Biervliet, J. P. G. M., Kamerling, J. P. & Wadman, S. K. (1977) D-Lactic aciduria, an inborn error of metabolism? Clin. Chim. Acta **74**, 297-300
3.2 Kandler, O. (1982) Gärungsmechanismen bei Milchsäurebakterien, Forum Mikrobiologie **5**, 16–22
3.3 Giesecke, D., Stangassinger, M. & Henle, K. (1985) D(–)-Milchsäure – ein Stoffwechselproblem, Zeitschrift für Ernährungswissenschaft **24**, 172–186
3.4 Barth, C. A. & de Vrese, M. (1984) D-Lactat im Stoffwechsel des Menschen – Fremdstoff oder physiologischer Metabolit? Kieler Milchwirtschaftliche Forschungsberichte **36**, 155–161
3.5 de Vrese, M., Koppenhoefer, B. & Barth, C. A. (1990) D-Lactic acid metabolism after an oral load of DL-lactate, Clinical Nutrition **9**, 23–28
3.6 Bässler, K. H. (1988) Die physiologische Rolle von Laktat im Licht neuerer Erkenntnisse, Ernährungs-Umschau **35**, 71–74
4.1 Mayer, K. & Pause, G. (1969) Enzymatische Milchsäurebestimmung in Weinen, Mitt. Geb.Lebensm.Unters.Forsch. **60**, 230–233
4.2 Postel, W. Drawert, F. & Hagen, W. (1973) Enzymatische Untersuchungen über den Gehalt an L(+)- und D(–)-Lactat in Weinen, Z.Lebens.-Unters.u.Forsch. **150**, 267–273
4.3 Bandion, F. & Valenta, M. (1977) Zur Beurteilung des D(–)- und L(+)-Milchsäuregehalts in Wein, Mitt. Klosterneuburg **27**, 4–10
4.4 Drawert, F. & Hagen, W. (1970) Enzymatische Analysenmethoden zur Bestimmung von Würze- und Bier-Inhaltsstoffen, I. Bestimmung von L(+)- und D(–)-Milchsäure in Bier, Brauwissenschaft **23**, 1–6
4.5 Piendl, A. & Wagner, I. (1983) Physiologische Eigenschaften der organischen Säuren des Bieres; 3. L(+)-Lactat und D(–)-Lactat, Brauindustrie **68**, 1520–1528
4.6 Steffen, Chr. (1971) Methoden zur Bestimmung der Gesamtmilchsäure und der Lactatkonfiguration in Käse und Milch, Schweiz.Milchzeitung **97**, 1073–1078
4.7 Steffen, Ch., Nick, B. & Blanc, B. H. (1973) Konfiguration der Milchsäure verschiedener Milchsäurebakterienstämme in Abhängigkeit fabrikationstechnischer Bedingungen, Schweiz. Milchw. Forsch. **2**, 37–52
4.8 Zaadhof, K.-J., Kohler, J. & Terplan, G. (1975) Erhebungen über den L(+)- und D(–)-Lactat- sowie Pyruvatgehalt in Trockenmilchprodukten, Deutsche Molkerei-Zeitung F.9, 228–233
4.9 Kielwein, G. & Daun, U. (1979) Vorkommen und Bedeutung von D(–)-Milchsäure in fermentierten Milcherzeugnissen unter besonderer Berücksichtigung von Mischerzeugnissen auf Joghurtbasis, Deutsche Molkerei-Zeitung **30**, 290–293
4.10 Jager, H.H. & Tschager, E. (1980) Die enzymatische Bestimmung von L-Milchsäure, D-Milchsäure, Glutaminsäure, Essigsäure und Brenztraubensäure im Käse, Milchwirtschaftliche Berichte **64**, 235–240
4.11 Schiweck, H. & Büsching, L. (1972) Die Bildung von D- und L-Milchsäure während des Fabrikationsprozesses, Zucker **25**, 7–12
4.12 van der Poel, P. W. (1975) Die mikrobiologische Überwachung des Extraktionssystems durch die Milchsäurebestimmung, Zucker **28**, 295–298
4.13 Kubadinow, N. & Rösner, G. (1977) Die enzymatische Bestimmung von D- und L-Milchsäure in Betriebssäften der Zuckerproduktion, ZUCKER **30**, 420–426
4.14 Sinell, H.-J. & Luke, K. (1979) D(–)-Lactat als Parameter für die mikrobielle Belastung von vakuumverpacktem Brühwurstaufschnitt, Fleischwirtschaft **59**, 547–550
4.15 Schneider, W., Hildebrandt, G. & Sinell, H.-J. (1983) D(–)-Lactat-Konzentration als Parameter für die Bewertung des Frischezustandes vorverpackter, hitzebehandelter Fleischerzeugnisse, Fleischwirtschaft **63**, 1198–1205
4.16 Spicher, G. & Rabe, E. (1981) Die Mikroflora des Sauerteigs (XII. Mitteilung), Z. Lebensm. Unters. Forsch. **172**, 20–25
4.17 Klopper, W. J., Angelino, S.A.G.F., Tuning, B. & Vermeire, H.A. (1986) Organic acids and glycerol in beer, J.Inst.Brew. **92**, 225–228
4.18 Stoya, W., Wachendörfer, G., Kary, I., Siebentritt, P. & Kaiser, E. (1987) Milchsäure als Therapeutikum gegen Varroatose und ihre Auswirkung auf den Honig, Deutsche Lebensmittel-Rundschau **83**, 283–286
4.19 Liepe, H.-U. (1987) Milchsaure Gemüsesäfte mit Starterkulturen, Flüssiges Obst **54**, 380–381
4.20 Littmann-Nienstedt, S. & Beutler, H.-O. (1988) Auswertung des Ringversuches der Arbeitsgruppe „Ei-Analytik" zur Bestimmung von L(–)-Milchsäure, 3-Hydroxybuttersäure und Bernsteinsäure in Eiprodukten, Deutsche Lebensmittel-Rundschau **84**, 320–322
4.21 Franzke, Cl. & Kroll, J. (1980) Zur enzymatischen Milchsäure-Bestimmung in Emulgatoren, Die Nahrung **24**, 89–90
5.1 Henniger, G. & Boos, H. (1978) Anwendung der enzymatischen Analyse bei der Untersuchung kosmetischer Präparate – dargestellt an einigen Beispielen, Seifen – Öle – Fette – Wachse **104**, 159–164
5.2 Stegmann, H. & Kindermann, W. (1981) Bestimmung der individuellen anaeroben Schwelle bei unterschiedlich Ausdauertrainierten aufgrund des Verhaltens der Lactat-Kinetik während der Arbeits- und Erholungsphase, Deutsche Zeitschrift für Sportmedizin **32**, 213–221
5.3 Kleine, T. O. (1991) D-Lactat und L-Lactat im Liquor cerebrospinalis bei akuten entzündlichen Erkrankungen des Zentralnervensystems (ZNS), Lab.med. **15**, 114–116

L-Lactic acid standard solution

Concentration: see bottle label
The L-lactic acid standard solution is a stabilized aqueous solution of L-lactic acid. It serves as a standard solution for the enzymatic analysis of L-lactic acid in foodstuffs and other materials.

Application
1. Addition of L-lactic acid standard solution to the assay
Instead of sample solution the standard solution is used for the assay.

2. Restart of the reaction, quantitatively:
After completion of the reaction with sample solution and measuring of A_2, add 0.050 ml standard solution to the assay mixture. Read absorbance A_3 after the end of the reaction (approx. 30 min). Calculate the concentration from the difference of $(A_3–A_2)$ according to the general equation for calculating the concentration. The altered total volume must be taken into account. Because of the dilution of the assay mixture by addition of the standard solution, the result differs insignificantly from the data stated on the bottle label.

3. Internal standard
The standard solution can be used as an internal standard in order to check the determination for correct performance (gross errors) and to see whether the sample solution is free from interfering substances:

Pipette into cuvettes	Blank	Sample	Standard	Sample + standard
solution 1	1.000 ml	1.000 ml	1.000 ml	1.000 ml
solution 2	0.200 ml	0.200 ml	0.200 ml	0.200 ml
suspension 3	0.020 ml	0.020 ml	0.020 ml	0.020 ml
sample solution	–	0.100 ml	–	0.050 ml
standard solution	–	–	0.100 ml	0.050 ml
redist. water	1.000 ml	0.900 ml	0.900 ml	0.900 ml
Mix, and read absorbances of the solutions (A_1) after approx. 5 min. Continue as described in the pipetting scheme under "Procedure". Follow the instructions given under "Instructions for performance of assay" and the footnotes.				

The recovery of the standard is calculated according to the following formula:

$$\text{recovery} = \frac{2 \times \Delta A_{\text{sample + standard}} - \Delta A_{\text{sample}}}{\Delta A_{\text{standard}}} \cdot 100\ [\%]$$

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