

# L-Lactic acid

## UV-method

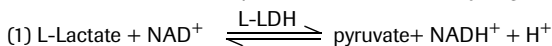
for the determination of L-lactic acid in foodstuffs and other materials

**Cat. No. 10 139 084 035**

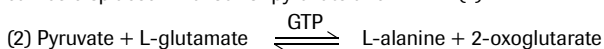
Test-Combination for 30 determinations

### Principle (Ref. 1)

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



The equilibrium of this reaction lies on the side of L-lactate. 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).



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

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

### Preparation of solutions

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

### Stability of reagents

The contents of bottles 1, 2, 3 and 4 are stable at 2-8°C (see pack label).

Bring solution 1 to 20-25°C before use.

Solution 2 is stable for 3 weeks at 2-8°C, and for 2 months at -15 to -25°C.

### Procedure

Wavelength<sup>1</sup>: 340 nm, Hg 365 nm or Hg 334 nm  
 Glass cuvette<sup>2</sup>: 1.00 cm light path  
 Temperature: 20-25°C  
 Final volume: 2.240 ml  
 Read against air (without a cuvette in the light path) or against water or against blank<sup>3</sup>  
 Sample solution: 0.3-35 µg of L-lactic acid/assay<sup>4</sup> (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 <sub>1</sub> ) after approx. 5 min. Start reaction by addition of:		
solution 4	0.020 ml	0.020 ml
Mix**, after completion of the reaction (approx. 30 min) read absorbances of blank and sample (A <sub>2</sub> ) 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 (trademark of the American Can Company, Greenwich, Ct., USA)

## BOEHRINGER MANNHEIM / R-BIOPHARM Enzymatic BioAnalysis / Food Analysis

For use in *in vitro* only

Store at 2-8°C

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

Determine the absorbance differences (A<sub>2</sub>-A<sub>1</sub>) for both, blank and sample. Subtract the absorbance difference of the blank from the absorbance difference of the sample.

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

The measured absorbance differences should, as a rule, be at least 0.100 absorbance units to achieve sufficiently precise results (see "Instructions for performance of assay" and "Sensitivity and detection limit", pt. 4).

### Calculation

According to the general equation for calculating the concentration:

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

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:

$$\begin{aligned} 340 \text{ nm} &= 6.3 \text{ [l} \times \text{mmol}^{-1} \times \text{cm}^{-1}] \\ \text{Hg } 365 \text{ nm} &= 3.4 \text{ [l} \times \text{mmol}^{-1} \times \text{cm}^{-1}] \\ \text{Hg } 334 \text{ nm} &= 6.18 \text{ [l} \times \text{mmol}^{-1} \times \text{cm}^{-1}] \end{aligned}$$

It follows for L-lactic acid:

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

If the sample has been diluted on 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 assay has to be between 0.5 µg and 35 µg (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 measurement at		Dilution with water	Dilution factor F
340 or 334 nm	365 nm		
< 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

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 to obtain the same final volume in the assays for sample and blank. The new sample volume v must be taken into account in the calculation.

1 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.

2 If desired, disposable cuvettes may be used instead of glass cuvettes.

3 For example, when using a double-beam photometer

4 See instructions for performance of assay

## 2. Technical information

- Perspiration of the hands contains L-lactic acid. Therefore, care should be taken not to touch the tips of the pipettes with fingers.
- 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 89.1g/ mol). (In enzymatic determinations, the L-lactate ion is measured.)
- 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.
- 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 after 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).
- Commercial lactic acid may not contain the stereo-isomeric 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 assay control solutions. The lithium and also the calcium salts are suited for the use as assay control.

## 3. Specificity (Ref. 1)

The method is specific for L-lactic acid.

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

## 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 1.5mg/l sample solution).

The detection limit 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 approx. 0.3  $\mu\text{g}$  L-lactic acid/assay (0.3 mg L-lactic acid/l sample solution; sample volume  $v = 1.000$  ml) to 35  $\mu\text{g}$  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:

CV = 2.3 %	lithium-L-lactate solution	(Ref. 1.3)
Yohurt: $x = 0.6$ g/100 g	$r = 0.05$ g/100 g	$R = 0.07$ g/100 g
Milk powder: $x = 0.112$ g/100 g	$r = 0.008$ g/100 g	$R = 0.015$ g/100 g
Whole egg powder: $x = 240$ mg/kg	$r = 62.8$ mg/kg $R = 89.1$ mg/kg	$s_{(f)} = \pm 22.2$ mg/kg $s_{(R)} = \pm 31.5$ mg/kg (Ref. 2.1)
For further data see references		
Wine:	$r = 0.02 + 0.07 \times x_i$ $x_i = \text{L-lactic acid concentration in g/l}$	$R = 0.05 + 0.125 \times x_i$ (Ref. 2.14, 2.15)

## 7. Interferences/sources of error

Traces of glutamate dehydrogenase (GIDH) in GPT result in reagent-dependent creep reactions. An extrapolation of  $A_2$  is not necessary if the absorbances of blank and sample are read immediately one after the other, and if there is no additional sample dependent creep reaction. See also pt. 2.4.

## 8. Recognizing interference during the assay procedure

- If the conversion of L-lactic acid has been completed according to the time given under "Procedure", it can be concluded in general that no interference has occurred.
- 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.
- 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.
- 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** assay control solution in the same assay. The recovery can then be calculated from the absorbance differences measured.
- Possible losses during the determination can be recognized by carrying out recovery tests: the sample should be prepared and analyzed with and without added standard material. The additive should be recovered quantitatively within the error range of the method.

## 9. 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.

## 10. 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 pH 8-10 by adding sodium or potassium hydroxide solution;

Adjust **acid and weakly colored samples** to 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 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 polyvinylpyrrolidone (PVPP) or with polyamide, e.g. 1g/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;

Deproteinize **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 the liquid sample into a 100 ml volumetric flask which contains approx. 60 ml redist. water, 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 mM = 3.60 g  $\text{K}_4[\text{Fe}(\text{CN})_6] \times 3 \text{H}_2\text{O}/100 \text{ ml}$ ) and 5 ml Carrez-II-solution (zinc sulfate, 250 mM = 7.20 g  $\text{ZnSO}_4 \times 7 \text{H}_2\text{O}/100 \text{ ml}$ ). Adjust to pH 7.5-8.5 with sodium hydroxide (0.1 M; e.g. 10 ml). Mix 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. 11 (application examples). Note: Treatment with concentrated Carrez-solutions has proved beneficial in routine analysis. In Germany, the method has been standardized and published in the official methods collection acc. to § 35 of the Foodstuffs and Consumer Goods Law (Lebensmittel- und 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.**

## 11. 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 polyvinylpyrrolidone (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 and mix. For clarification, add 5 ml Carrez-I-solution (3.60 g potassium hexacyanoferrate(II),  $K_4[Fe(CN)_6] \times 3 H_2O/100$  ml), 5 ml Carrez-II-solution (7.20 g zinc sulfate,  $ZnSO_4 \times 7 H_2O/100$  ml) and 10 ml NaOH (0.1 M), after each addition fill up with water to the mark, mix and filter. Use the clear, possibly slightly turbid solution for the assay, diluted if necessary.

### 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 M) for 15 min under a reflux condenser, allow to cool to 20-25°C and neutralize with sulfuric acid (1 M; use indicator paper). Transfer solution quantitatively into a 50 ml volumetric flask and fill up to the mark with water and mix. 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<sub>2</sub>-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<sub>2</sub>.

### Determination of L-lactic acid in sauerkraut juice

Transfer 1.00 ml of sauerkraut juice into a 100 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 yogurt (milk)

Mix 2 g of yogurt (milk) with 98 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 20-25°C 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 M) 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 M) 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 20-25°C, and add one after the other and shake after each addition: 1 ml concentrated Carrez-I-solution (15.0 g potassium hexacyanoferrate(II),  $K_4[Fe(CN)_6] \times 3 H_2O/100$  ml), 1 ml concentrated Carrez-II-solution (30.0 g zinc sulfate,  $ZnSO_4 \times 7 H_2O/100$  ml). Fill up to the mark with NaOH (0.1 M), mix and filter with fluted filter paper and glass funnel. 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 20-25°C, add one after the other and shake rigorously after each addition: 1 ml concentrated Carrez-I-solution (15.0 g potassium hexacyanoferrate(II),  $K_4[Fe(CN)_6] \times 3 H_2O/100$  ml), 1 ml concentrated Carrez-II-solution (30.0 g zinc sulfate,  $ZnSO_4 \times 7 H_2O/100$  ml). Adjust to pH 8-9 with NaOH (1 M), fill up to the mark with redist. water, mix and filter with fluted filter paper and glass funnel. Use 0.100-0.500 ml filtrate for the assay. The altered sample volume must be taken into account in the calculation.

## 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 M) for 10 min under a reflux condenser. Allow solution to cool to 20-25°C, and neutralize or acidify slightly, respectively, with approx. 5 ml HCl (5 M). 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-lactic acid. 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.

### 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

- 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
- Noll, F. (1966) Methode zur quantitativen Bestimmung von L-(+)-Lactat mittels Lactat-Dehydrogenase und Glutamat-Pyruvat-Transaminase, Biochem.Z. **346**, 41-49
- 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
- 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
- Ämtliche Sammlung von Untersuchungsverfahren nach §35 LMBG; Untersuchung von Lebensmitteln: Bestimmung von L- und D-Milchsäure (L- und D-Lactat) in Fleisch-erzeugnissen, 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)
- Schweizerisches Lebensmittelbuch, Kapitel 61B (Enzymatische Bestimmungen)/3.3. (1981), Kapitel 1 (Milch)/5.2 (1987), Kapitel 2B (Säuermilchprodukte)/18 (1980), Kapitel 4 (Milchdauerwaren)/10.4. (1993), Kapitel 28A (Frucht- und Gemüsesäfte u.a.)/7.5

(1988), Kapitel 30A (Wein aus Trauben)/6.4. (1993), Kapitel 34 (Gärungssessig)/4.4 (1994)

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

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

2.5 International Federation of Fruit Juice Producers (IFU, Methods of Analysis, no. 53-1983); contained in "Code of Practice for Evaluation of Fruit and Vegetable Juices" (1996) edited by Association of the Industry of Juices and Nectars from Fruits and Vegetables of the European Economic Community (A.I.J.N.)

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

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) Approvazione 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 betreft 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 DIN 10335 (Juni 1987) Bestimmung des Gehaltes an L- und D-Milchsäure (L- und D-Lactat) in Milch und Milchprodukten, Enzymatisches Verfahren

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 DELA 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

2.17 Deutsche Norm DIN EN 12631 (April 1999) Frucht- und Gemüsesäfte: Enzymatische Bestimmung des Gehaltes an D- und L-Milchsäure (Lactat)

2.18 Europäische Norm / European Standard EN 12631 (April 1999) Frucht- und Gemüsesäfte: Enzymatische Bestimmung des Gehaltes an D- und L-Milchsäure (Lactat) - Spektralphotometrische Bestimmung von NAD (Fruit and vegetable juices - Enzymatic determination of D- and L-lactic acid (lactate) content - NAD spectrometric method)

2.19 Standard der Russischen Föderation / Standard of the Russian Federation / GOSSTANDART ROSSII GOST R 51196-98 (1998) Dried milk. Method for determination of lactic acid and lactates

3.1 Kandler, O. (1982) Gärungsmechanismen bei Milchsäurebakterien, Forum Mikrobiologie **5**, 16-22

3.2 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 Analysemethoden 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 Molkezeitung F.9, 228-233

4.9 Kielwein, G. & Daun, U. (1979) Vorkommen und Bedeutung von D(-)-Milchsäure in fermentierten Milcherzeugnissen unter besonderer Berücksichtigung von Misch-erzeugnissen auf Joghurtbasis, Deutsche Molkezeitung **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, Milch-wirtschaftliche 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 Betriebsäften der Zuckerproduktion, ZUCKER **30**, 420-426

4.14 Spicher, G. & Rabe, E. (1981) Die Mikroflora des Sauerteigs (XII. Mitteilung), Z. Lebensm. Unters. Forsch. **172**, 20-25

4.15 Klopfer, 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

4.16 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 **18**, 283-286

4.17 Lieve, H.-U. (1987) Milchsäure Gemüsesäfte mit Starterkulturen, Flüssiges Obst 380-381

4.18 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

4.19 Franzke, Cl. & Kroll, J. (1980) Zur enzymatischen Milchsäure-Bestimmung in Emulgatoren, Die Nahrung **24**, 89-90

4.20 Schlimme, E., Lorenzen, P. Chr., Martin D. & Thormählen, K. (1996) Analytical differentiation of butter types by specific compositional parameters of the aqueous butter-phase, Milchwissenschaft **51**, 139-143

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

# L-Lactic acid assay control solution (Bottle 5)

**Concentration:** see bottle label

The L-lactic acid assay control solution is a stabilized aqueous solution of L-lactic acid. It serves as an assay control solution for the enzymatic determination of L-lactic acid in foodstuffs and other materials.

## Application:

### 1. Addition of L-Lactic acid assay control solution to the assay mixture:

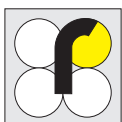
Instead of sample solution the assay control solution is used for the assay.

### 2. Restart of the reaction, quantitatively:

After completion of the reaction with sample solution and measuring of  $A_2$ , add 0.050 ml assay control 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 assay control solution, the result differs insignificantly from the data stated on the bottle label.

## Also Available:

**Test-Combination D-Lactic acid/L-Lactic acid,  
Cat. No. 11 112 821 035**



R-BIOPHARM AG  
Landwehrstr. 54  
D-64293 Darmstadt  
Telefon + 49 61 51 / 81 02-0  
Fax + 49 61 51 / 81 02-20  
www.r-biopharm.com

## 3. Internal standard:

The assay control 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
assay control sln.	-	-	0.100 ml	0.050 ml
recist. 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:

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

