UV method for approx. 32 assays

For laboratory use only
Store between +2 and +8°C

The methods (D- and/or L-lactic acid) are contained in the Dutch, German, Italian, Swiss food laws and in European regulations. Recommended e. g. by IDF, IFU, AIJN, MEBAK, OIV, VDLUFA. Standardized by DIN, EN, ISO, GOST.

Principle

L-Lactate + NAD^+ ——— L-LDH ⟶ pyruvate + NADH + H^+

Pyruvate + L-glutamate ——— GPT ⟶ L-alanine + 2-oxoglutarate

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

Assay performance

Wavelength: 340 nm (NADH), ε = 6.3 l x $mmol^{-1}$ x cm^{-1}
Light path: 1.00 cm (glass or plastic cuvettes)
Temperature: +20 to +25°C
Assay volume: 2.240 ml
Measurement: against air or against water
Sample solution: 0.3 to 30 µg L-lactic acid in 0.100 to 1.000 ml sample solution.

Reagents

1: Approx. 34 ml glycylglycine buffer, pH approx. 10.0, approx. 490 mg L-glutamic acid (for stability see pack label). *The solution is ready for use*.

2: NAD, lyophilizate, approx. 250 mg (for stability see pack label). *Dissolve contents of bottle # 2 with 7 ml redist. water*. The solution is stable for 1 month at +2 to +8°C, resp. for 2 month s at -15 to -25°C.

3: Approx. 0.7 ml glutamate pyruvate transaminase (GPT) suspension (approx. 1100 U) in ammonium sulfate (for stability see pack label). *The suspension is ready for use*. Swirl bottle carefully before the suspension is pipetted.

4-L: Approx. 0.7 ml L-lactate dehydrogenase (L-LDH) solution (approx. 3800 U) in glycerol (for stability see pack label). *The solution is ready for use*.

In addition (not contained in the kit):

Standard solution L-lactate, lithium or calcium salt, 0.15 g L-lactic acid/l for test control only.

The reagents for the determination of L-lactic acid are not hazardous. The general safety rules for the work in chemical laboratories should be applied. After use the reagents can be disposed of with the laboratory waste. Packaging materials may be recycled.

Procedure

Pipette into cuvettes:	**Blank**	**Standard assay[1]**	**Sample assay[2]**	**Rerun assay[3]**	**Assay with internal standard[4]**	**High sensitive assay[5]**
Glycylglycine buffer # 1	1.000 ml	1.000 ml	1.000 ml	1.000 ml	1.000 ml	1.000 ml
NAD solution # 2	0.200 ml	0.200 ml	0.200 ml	0.200 ml	0.200 ml	0.200 ml
GPT suspension # 3	0.020 ml	0.020 ml	0.020 ml	0.020 ml	0.020 ml	0.020 ml
Sample solution[6] (e.g. 0.02 to 0.15 g L-lactic acid/l)	**-**	**-**	**0.100 ml**	**0.200 ml**	**0.100 ml**	**1.000 ml**
Standard solution[6] (e.g. 0.15 g L-lactic/l)	-	0.100 ml	-	-	0.100 ml	-
Redist. Water	1.000 ml	0.900 ml	0.900 ml	0.800 ml	0.800 ml	-
Mix[7], after approx. 5 min read the absorbances (A_1). Add:						
L-LDH solution # 4-L	0.020 ml	0.020 ml	0.020 ml	0.020 ml	0.020 ml	0.020 ml
Mix[7], after completion of the reaction (approx. 30 min) read the absorbances of blank and the other assays (A_2) immediately one after the other. Repeat absorbance reading after another 2 min[8].						

Notes

1 Run a standard to see accidents in analysis. The measurement of the standard is not necessary for calculating results.
2 This assay together with the blank is a single determination.
3 In the case of a double determination, run two assays with different sample volumes. The absorbance differences measured have to be proportional to the sample volumes (calculate with resp. volume v).
4 Recovery = [(Δ $A_{sample+standard}$ − Δ A_{sample}) / Δ $A_{standard}$] x 100 [%].
5 Assay recommended in the case of trace level compound analysis, with sample volume up to 1.000 ml (0.0003 to 0.015 g/l L-Lactic acid).
6 Before dispensing, rinse the enzyme pipette, resp. the tip of the piston pipette with sample resp. with standard solution
7 e.g. with a plastic spatula, or after closing the cuvette with Parafilm (trademark of American Can Co., Greenwich Ct., USA)
8 The reaction has stopped when the absorbance is constant. If the reaction has not stopped, continue to read absorbances until they increase constantly over e. g. 2 min. Extrapolate absorbances to the time of the addition of L-LDH (solution # 4-L).

Calculation[9]

$\Delta A_{L\text{-lactic acid}} = (A_2 - A_1)_{sample,\ resp.\ standard} - (A_2 - A_1)_{blank}$

C = (V x MW x Δ A) / (ε x d x v x 1000) [g L-lactic acid /l sample solution]

c = (2.240 x 90.1 x Δ A) / (6.3 x 1.00 x 0.100 x 1000) = **0.3204 x Δ A [g L-lactic acid / l sample solution]**

If the sample has been diluted during preparation, multiply the result with the dilution factor F.

When analyzing samples which are weighed out for sample preparation, calculate the content from the amount weighed:

$$Content_{L\text{-lactic acid}} = \frac{C_{L\text{-lactic acid}}\ [g/l\ sample\ solution]}{weight_{sample}\ [\ in\ g/l\ sample\ solution]} \times 100\ [g/100\ g]$$

Sample preparation

If the sample has one of the characteristics below, which hamper the test, please follow the corresponding sample preparation procedure.

1. Dilute *clear, colourless and almost neutral liquid samples* to get a sample solution with 0.02 to 0.15 g L-lactic acid /l.
2. Filter or centrifuge *turbid solutions*, dilute (see pt. 1).
3. Degas *samples containing carbon dioxide*, e.g. by filtration, or add $NaHCO_3$ until the solution is slightly alkaline, dilute (see pt. 1).
4. Adjust acid (esp. slightly coloured) solutions with KOH or NaOH to approx. pH 8 to 10, incubate a few minutes, or dilute (see pt. 1) without pH adjustment in the case of colourless samples.
5. Measure *coloured samples* (adjusted to pH 8) against a sample blank.
6. Treat *strongly coloured solutions* used undiluted with PVPP or polyamide, e.g. 1 g/100 ml, mix, incubate a few minutes, filter.
7. Crush (corn size < 0.3 mm) or homogenize *solid or semi-solid (pasty) samples*, extract with water or dissolve in water, filter and dilute (see pt. 1) if necessary.
8. Extract *fat containing samples* with hot water at a temperature above the melting point of fat, e.g. in a 100 ml volumetric flask. Adjust to +20 ℃, fill volumetric flask to the mark. Store in ice or in refrigerator for approx. 15 resp. 30 min, filter. Alternatively, clarify with Carrez reagents (which can be recommended).
9. Clarify *samples containing protein* with Carrez reagents:
   Weigh sufficient quantity of solid or pasty sample into 100 ml volumetric flask, add approx. 60 ml water. Or pipette liquid sample into 100 ml volumetric flask containing approx. 60 ml water. Add, and mix after each addition, 5 ml Carrez-I-solution (3.60 g $K_4[Fe(CN)_6]$ x $3H_2O$ = potassium hexacyanoferrate(II)/100 ml), 5 ml Carrez-II-solution (7.20 g $ZnSO_4$ x 7 H_2O = zinc sulfate hepta-hydrate/100 ml). Adjust to pH 7.5 to 8.5 by the addition of e.g. 10 ml NaOH (0.1 M). Fill the flask to the mark, mix and filter.
10. Deproteinize *samples containing protein* with perchloric acid.

Assay characteristics

1. *Specificity:* Specific for L-lactic acid (the L-lactate ion reacts). In the analysis of commercial L-lactate results of approx. 98% have to expected. Free L-lactic acid cannot be used as a standard material because the partially formed lactyl-lactate does not react.
2. *Sensitivity:* 0.15 mg /l (ΔA = 0.005; v = 1.000 ml; V = 2.240 ml)
3. *Detection limit*: 0.3 mg /l (ΔA = 0.010; v = 1.000 ml; V = 2.240 ml)
4. *Linearity*: from 0.3 µg /assay (v = 1.000 ml; V = 2.240 ml)
   to 30 µg /assay (v = 0.100 ml; V = 2.240 ml)
5. *Precision:* ΔA = +/- 0.005 to +/- 0.010 absorbance units
   CV = approx. 2 to 3 %
   Yogurt: x = 0.6 g/100g
   r = 0.05 g/100 g
   R = 0.07 g/100 g
   Wine: $r = 0.02 + 0.07 \times c_{L\text{-lactic acid}}$ [g/l]
   $R = 0.05 + 0.125 \times c_{L\text{-lactic acid}}$ [g/l]
6. *Interferences*: There may be a reagent dependent creep reaction after the conversion of L-lactate at A_2. An extrapolation of the absorbances back to the time of the addition of L-LDH (solution # 4-L) may not be necessary when the absorbances of blank and sample assays are measured immediately one after the other.
7. *Technical Information*: The reagents can also be used for the determination of D-lactic acid (with D-LDH instead of L-LDH). Perspiration of the hands contains L-lactate.

Notes

9 Results from acidimetric determination of 'total acid' (protons are measured) calculated as lactic acid cannot be compared with enzymatic analysis.