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

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

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

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

Ref.: Gawehn, K. (1984) in Methods of Enzymatic Analysis (Bergmeyer, H.U., ed.) 3rd. ed., vol. VI, pp. 588-592, Verlag Chemie, Weinheim, Basel. Noll, F. (1966) Methode zur quantitativen Bestimmung von L(+)-Lactat mittels LDH und GPT, Biochem. Z. 346, 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 volumes	(when the D-lactate content is smaller than the L-lactate content):
	2.240 ml (D-lactic acid)
	2.260 ml (L-lactic acid)
Measurement:	against air or against water
Sample solution:	0.3 to 30 µg D- + 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-D: Approx. 0.7 ml D-lactate dehydrogenase solution (approx. 3800 U) in glycerol (for stability see pack label). *The solution is ready for use.*

4-L: Approx. 0.7 ml L-lactate dehydrogenase 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 D-lactate, resp. L-lactate, lithium or calcium salts, 0.15 g D-lactic acid, resp. L-lactic acid/l for test control only.

The reagents for the determination of D- and 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 D- + L-lactic acid/l)	**-**	**-**	**0.100 ml**	**0.200 ml**	**0.100 ml**	**1.000 ml**
Standard solution[6] (e.g. 0.15 g D-, resp. L-lactic acid/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:						
D-LDH solution # 4-D	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. 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_3) immediately one after the other. Repeat absorbance reading after another 2 min[8].						

See notes on the next page

Calculation[9]

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

$\Delta A_{L\text{-lactic acid}} = (A_3 - A_2)_{\text{sample resp. standard}} - (A_3 - A_2)_{\text{blank}}$

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

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

c = (2.260 x 90.1 x ΔA) / (6.3 x 1.00 x 0.100 x 1000) = **0.3232 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:

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

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, colorless and almost neutral liquid samples* to get a sample solution with 0.02 to 0.15 g D- + 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 $NaHCO_3$ till the solution is slightly alkaline.
4. Adjust *acid (esp. slightly colored) 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 colorless samples.
5. Measure *"colored samples"* (adjusted to pH 8) against a sample blank.
6. Treat *"strongly colored 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°C, fill volumetric flask to the mark. Store in ice or in refrigerator for approx. 15 resp. 30 min, filter. Alternatively, clarify with Carrez reagents (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 with perchloric acid.

Assay characteristics

1. *Specificity:* Specific for D-lactic acid (the D-lactate ion reacts), resp. L-lactic acid (the L-lactate ion reacts). In the analysis of commercial D-lactate (L-lactate) results of approx. 99 (98) % have to be expected. Free lactic acid cannot be used as a standard material because the partially formed lactyl-lactate does not react.
2. *Sensitivity:* 0.15 mg /l (Δ A = 0.005; v = 1.000 ml; V = 2.240 / 2.260 ml)
3. *Detection limit*: 0.30 mg /l (Δ A = 0.010; v = 1.000 ml; V = 2.240 / 2.260 ml)
4. *Linearity*: from 0.3 µg /assay (v = 1.000 ml; V = 2.240 / 2.260 ml)
   to 30 µg /assay (v = 0.100 ml; V = 2.240 / 2.260 ml)
5. *Precision:* Δ A = ± 0.005 to ± 0.010 absorbance units
   CV = approx. 2 to 3 %
   Yogurt: r = 0.03 g D-lactic acid / 100g resp. 0.05 g L-lactic acid /100g
   R = 0.05 g D-lactic acid / 100g resp. 0.07 g L-lactic acid /100g
   Wine: $r = 0.02 + 0.07 \times c_{\text{L-lactic acid}}$ [g/l]
   $R = 0.05 + 0.125 \times c_{\text{L-lactic acid}}$ [g/l]
6. *Interferences*: There may be reagent dependent creep reactions after the conversion of lactate. An extrapolation of the absorbances back to the time of the enzyme addition may not be necessary, when the absorbances of blank and sample assays are measured immediately one after the other.
7. *Technical Information*: Perspiration of the hands contains L-lactate.

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 have to be proportional to the sample volumes. Calculate with the resp. volumes v.
4 Recovery = $[(\Delta A_{\text{sample+standard}} - \Delta A_{\text{sample}}) / \Delta A_{\text{standard}}] \times 100$ [%].
5 Assay recommended in the case of trace level analysis, with sample volume increased up to 1.000 ml (0.0003 to 0.015 g/l D + L-lactate).
6 Before dispensing, rinse the enzyme pipette, resp. the tip of the piston pipette with sample resp. with standard solution.
7 For example 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 until they increase constantly over e. g. 2 min. Extrapolate to the time of the addition of D-LDH (solution # 4-D) or L-LDH (solution # 4-L).
9 Results from acidimetric determination of 'total acid' calculated as lactic acid (protons are measured) cannot be compared with enzymatic analysis.