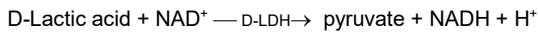


UV assay for the determination of D-lactic acid in foodstuffs and other sample materials
Test combination for 50 determinations

For *in vitro* use only
Store between 2 - 8 °C

1. Test principle

Enzymatic UV test for the quantitative determination of D-lactic acid with D-lactate-dehydrogenase (D-LDH).



D-Lactic acid reacts in the presence of nicotinamide-adenine-dinucleotide (NAD) and D-lactate-dehydrogenase (D-LDH) to pyruvate and NADH.

NAD is reduced to NADH. The amount of NADH is equimolar to the concentration of D-lactic acid. The development of the NADH and is measured at 340 nm.

For the determination of the sum of D- and L-lactic acid, the test Enzytec™ Liquid D-/L-Lactic acid (E8240) is suitable. If only the concentration of L-lactic acid is required, use Enzytec™ Liquid L-Lactic acid (E8260) or subtract the determined D-lactic acid concentration from the result of the test E8240. If only the concentration of D-lactic acid is required, use Enzytec™ Liquid D-Lactic acid (E8245) or subtract the determined L-lactic acid concentration from the result of the test E8240.

2. Reagents

2.1. Content & composition

The test is suitable for manual and automated processing. With manual processing, the reagents are sufficient for 50 determinations. The number of determinations for automated processing is increased by a multiple; however it depends on the device.

- Reagent 1: 2 x 50 mL with buffer, D-LDH
- Reagent 2: 2 x 12.5 mL with buffer, NAD

2.2. Reagent preparation

The reagents are ready-to-use and be allowed to reach room temperature (20 - 25 °C) before use. Do not interchange components between kits of different batches.

2.3. Storage & stability

The reagents are stable until the end of the month of the indicated shelf life (see label) even after opening at 2 - 8 °C if handled properly. Do not freeze reagents.

2.4. Safety & disposal

The general safety rules for working in chemical laboratories should be applied. Do not swallow! Avoid contact with skin and mucous membranes.

This kit may contain hazardous substances. For hazard notes on the contained substances, please refer to the appropriate safety data sheets (SDS) for this product. After use, the reagents can be disposed of with the laboratory waste. Packaging materials may be recycled.

3. Sample preparation

- Sample preparation for manual and automated testing is identical.
- The samples should be brought to room temperature before measurement.
- Use liquid, clear and almost neutral sample solutions directly or after dilution with dist. water to a concentration within the measuring range (see performance data).
- Filter or centrifuge turbid solutions.
- If necessary, decolorize strongly colored samples.
- Degas samples containing carbonic acid.
- Clarify protein-containing samples with Carrez reagents.
- Weigh samples with a high fat content into a volumetric flask and extract with hot water; allow sample solution to cool down for fat separation (e.g. 15 min in an ice bath); fill volumetric flask up to the mark with water, filter aqueous solution before testing.

4. Assays performance

Wavelength: 340 nm
Temperature: 20 - 37 °C (during the measurement)
Measurement: against air (without cuvette) or water
Measuring range: 15 - 500 mg/L

	Reagent blank	Samples / controls
Reagent 1	2000 µL	2000 µL
Sample / control	-	100 µL
Dist. water	100 µL	-
Mix, incubate for 3 min at 20 - 37 °C. Read absorbance A ₁ , then addition of:		
Reagent 2	500 µL	500 µL
Mix, incubate for 15 min at 20 - 37 °C and read absorbance A ₂ .		

The reagent blank value must be determined once for each run and subtracted from each sample result.

5. Calculation of results

5.1. Calculation of sample solutions

5.1.1. Concentration of D-lactic acid

$$\Delta A = (A_2 - df \times A_1)_{\text{sample}} - (A_2 - df \times A_1)_{\text{RB}}$$

df: Dilution factor
RB: Reagent blank

$$df = \frac{\text{sample volume} + R1}{\text{test volume}} = 0.808$$

Increasing the sample volume (up to max. 1000 µL) with unchanged reagent volumes requires conversion of the reagent dilution factor (df). If the volume is increased, the test system may be affected. In general, this must be checked depending on the matrix.

$$C_{\text{D-lactic acid}} [\text{g/L}] = \frac{(V \times MW \times \Delta A)}{(\epsilon \times d \times v \times 1000)} = 0.3718 \times \Delta A$$

V: Test volume basic application [mL] = 2.600
MW: Molecular weight [g/mol] = 90.08
d: Optical path [cm] = 1.00
v: Sample volume [mL] = 0.100
ε: Extinction coefficient NADH [L/mmol x cm] = 6.3 (at 340 nm)

5.2. Calculation of solid samples

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

5.3. Controls & acceptance criteria

Controls or reference samples should be carried along for quality control during each run. For this purpose, we recommend the use of Enzytec™ Liquid Multi-Acid Standard low (E8460).

The recovery of Enzytec™ Liquid Multi-Acid Standard low and other aqueous control solutions should be within 100 ± 5 %.

6. Performance data

6.1. Specificity

The test is specific for D-lactic acid.

6.2. Interferences

Oxalic acid showed no interferences below 0.2 g/L.

For ascorbic acid and 3-hydroxybutyric acid no interferences up to 0.2 g/L were found. For sulfite no interferences up to 0.05 g/L were found.

6.3. Linearity, measuring range & sensitivity

Linearity is given up to 500 mg/L D-lactic acid (sample volume of 100 µL).

The limit of detection (LoD) was determined for a sample volume of $v = 100 \mu\text{L}$ according to method DIN 32645:2008-11, using buffered aqueous solutions. This results in an LoD of 5 mg/L.

The limit of quantification (LoQ) was determined by precision profile and is 15 mg/L.

The smallest absorbance difference that the method can distinguish is $\Delta A = 0.005$. For a sample volume of $v = 1000 \mu\text{L}$, this results in an LoD of 0.25 mg/L. Based on $\Delta A = 0.010$, an LoQ of 0.50 mg/L was calculated.

6.4. Automation with Pictus 500

6.4.1. Limit of quantification (LoQ)

P500 application	LoQ
High Range	30 mg/L
Basic Range	5 mg/L
Sensitive Range	0.75 mg/L

6.4.2. Measuring ranges

P500 application	Measuring range
High Range	to 3.125 g/L
Basic Range	to 625 mg/L
Sensitive Range	to 62.5 mg/L

6.4.3. Precision and accuracy

Data from the measurement of an aqueous solution are shown here.

High Range

Target concentration, mg/L	150	450
Mean value, mg/L	152.2	451.8
SD, mg/L	2.87	6.04
RSD, %	1.88	1.34
Recovery, %	101.5	100.4

Basic Range

Target concentration, mg/L	150	450
Mean value, mg/L	149.4	449.8
SD, mg/L	1.02	2.89
RSD, %	0.69	0.64
Recovery, %	99.6	100.0

Sensitive Range

Target concentration, mg/L	15	45
Mean value, mg/L	15.13	44.31
SD, mg/L	0.15	0.24
RSD, %	1.02	0.54
Recovery, %	100.9	98.5

7. Supporting documents

On request, we offer the following documents:

- Enzytec™ Liquid Validation reports
- Enzytec™ Liquid Sample preparation guide
- Enzytec™ Liquid Excel templates for results calculation
- Enzytec™ Liquid Troubleshooting guide

Safety data sheets (SDS) und certificates of analysis (CoA) are available in digital form under the following link

<https://eifu.r-biopharm.com/>



8. Services & technical support

On request, we offer the following services:

- Customized troubleshooting
- Data & results analysis
- Customer workshops & webinars
- Automation: application support and technical service

9. Disclaimer

This information corresponds to our present state of technology and provides information on our products and their uses. R-Biopharm makes no warranty of any kind, either expressed or implied, except that the materials from which its products are made are of standard quality. Defective products will be replaced. There is no warranty of merchantability of this product, or of the fitness of the product for any purpose. R-Biopharm shall not be liable for any damages, including special or consequential damage, or expense arising directly or indirectly from the use of this product.