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

For *in vitro* use only
Store between 2 – 8 °C (36 – 46 °F)

This test was evaluated using selected samples of the following matrices: wine, milk and fermented milk products, fermented vegetable products, fruit and vegetable juices, beer, eggs, and egg powder.

Detailed results and information regarding associated validation data are found in the Validation Report.

The test has been approved as AOAC *Official Method of Analysis* 2024.07 First Action. A publication is available in J. AOAC Int. 108(4), 595–611 (2025).

The test may be used with other foods or samples material, provided that these are subjected to individual validation by the user.

1. Test principle

L-lactic acid (L-lactate) is oxidized by nicotinamide adenine dinucleotide (NAD) in the presence of the enzyme L-lactate dehydrogenase (L-LDH) to form pyruvate and NADH:

L-Lactate + NAD^+ $\xrightarrow{\text{L-LDH}}$ pyruvate + NADH + H^+

NAD is reduced to NADH during this process. The amount of NADH formed is proportional to the concentration of L-lactic acid in the sample and is measured at a wavelength of 340 nm.

The Enzytec™ *Liquid* D-/L-Lactic acid (E8240) assay can be used to determine the total concentration of D- and L-lactic acid. The concentration of D-lactic acid can also be determined directly using the Enzytec™ *Liquid* D-Lactic Acid (E8245) assay.

Alternatively, the single concentrations determined from the E8245 or E8260 tests can also be subtracted from the test result of the E8240 sum assay in order to differentiate and quantify the other lactic acid isomer.

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, L-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 / 68 – 77 °F) before use. Do not interchange components between kits of different batches.

2.3. Storage & stability

If stored as directed and between 2 – 8 °C (36 – 46 °F), reagents remain stable until the printed expiration date, even after opening. Reagents must not be frozen.

2.4. Safety & disposal

The test is intended solely for the intended use as described. The provided Instructions for Use must be strictly followed.

Follow standard chemical safety procedures when handling this product. Do not swallow. Avoid contact with skin or mucous membranes.

Detail safety information for individual components is available in the corresponding Safety Data Sheets (SDS).

Dispose of used reagents as laboratory waste in compliance with all relevant regulations. Packaging materials are to be recycled according to local regulations.

3. Sample preparation

- Sample preparation for manual and automated testing is the same.
- Samples solutions should be brought to room temperature before measurement.
- Use liquid, clear and almost neutral sample solutions directly or after dilution with distilled water to a concentration within the measuring range (see performance data).
- Neutralize **strongly** acidic samples (e.g., white wine and fruit juices if used undiluted) to a pH value between 6.5 and 7.5 by adding 1 M KOH.
- For turbid test samples (e.g., sauerkraut juice, pineapple juice): Filter by using fluted paper filter or syringe filter or centrifuge the test solution in a reaction tube (recommended 3000 rpm for at least 5 minutes) until a clear filtrate or supernatant is obtained. Use 100 µL (or more if necessary; fruit juices undiluted, fermented vegetable juice should be diluted before measurement).
- Degas samples containing carbon dioxide (e.g., beer) by aid of a short ultrasound burst (10s), filter if not clear, and use 100 µL of the undiluted extract.
- If necessary, decolorize strongly colored samples (such as wine and juices) with polyvinylpolypyrrolidone (PVPP, e.g., 1 g/100 ml sample). Stir or shake for 1 minute and filter or centrifuge at 3000 rpm for at least 5 minutes until a clear supernatant is obtained.
- Clarify samples containing proteins or fat with Carrez clarification: Weigh or pipette the appropriate sample quantity into a 100 mL volumetric flask and add approx. 60 mL distilled water. Then add 5 mL Carrez I solution (3.60 g potassium hexacyanoferrate(II)-trihydrate $K_4[Fe(CN)_6]$ 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 0.1 M NaOH. Mix well after each addition. Fill the measuring flask with distilled water up to the mark, mix and filter (discard first milliliters).
- Crush and homogenize solid and semi-solid samples and extract with water. Filter, centrifuge, or use Carrez clarification if necessary.
- For samples with a high fat content, weigh e.g. 5 g into a 100 mL volumetric flask, fill halfway with water, and heat in a water bath at 50 – 60 °C (122 – 140 °F) for 20 minutes. After cooling, fill the flask to the mark and place it in the refrigerator for about 20 minutes to separate the fat. Then use a pleated filter to obtain a clear or slightly cloudy sample.
- In case of higher sample volumes (up to 1000µL), check the pH value of the test solution and neutralize in case of any doubt.

3.1. Sausages and sausage products

- Weigh 5 g homogenized sample into a 50 mL Falcon tube.
- Add 20 mL 1 M perchloric acid, vortex to suspend and rotate for 10 minutes.
- Transfer to a beaker with approx. 40 mL distilled water; add 5 M KOH and stir until the pH value is approx. 7.
- Transfer to a 100 mL volumetric flask; dilute with distilled water to the mark, and store in a refrigerator at 2 – 8 °C (36 – 46 °F) for 20 minutes.
- Filter through a fluted paper filter and use 100 µL of the extract.

3.2. Yogurt and cream cheese

- Weigh 2 g of sample into a 100 mL volumetric flask.
- Add distilled water, shake to suspend, and dilute to the mark with water.
- In case of cream cheese, add 10 mL distilled water to 2 g of sample, suspend, add another 10 mL and repeat this procedure until the material is fully suspended.
- Then dilute to 100 mL with distilled water; filter through a fluted paper filter or centrifuge at 3000 rpm for at least 5 minutes until a clear supernatant is obtained.
- Use 100 µL sample solution for analysis.

3.3. Milk and tomato juice

- Pipet 1 mL milk or 5 mL tomato juice into a 50 mL volumetric flask.
- Add 10 mL distilled water, add 2.5 mL Carrez I solution, shake, and add 2.5 mL Carrez II solution; shake and add 5 mL 0.1 M NaOH.
- Shake and dilute with distilled water to the mark; filter through a fluted paper filter or centrifuge at 3000 rpm for at least 5 minutes until a clear supernatant is obtained.
- Use 1000 µL sample solution for analysis.

3.4. Cream and whole milk powder

- Weigh 2 g sample into a 100 mL volumetric flask.
- Add 10 mL distilled water, add 5 mL Carrez I solution, shake, and add 5 mL Carrez II solution; shake and add 10 mL 0.1 M NaOH.
- Shake and dilute with distilled water to the mark; filter through a fluted paper filter or centrifuge at 3000 rpm for at least 5 minutes until a clear supernatant is obtained.
- Use 1000 µL sample solution for analysis.

3.5. Whole egg powder

- Weigh 2 g sample into a 50 mL Falcon tube.
- Add 10 mL distilled water and one drop 1-octanol.
- Shake and incubate for 15 minutes in a boiling water bath; cool down to room temperature and transfer to a 50 mL volumetric flask.
- Add 2 mL concentrated Carrez I solution, shake, add 2 mL concentrated Carrez II solution, shake, and add 1 M NaOH until pH value is approx. 8 (usually about 200 µL 1 M NaOH are required).
- Dilute with distilled water to the mark, shake, and filter through a fluted paper filter.
- Use 1000 µL sample solution for analysis.

3.6. Liquid egg

- Weigh 5 g sample into a 50 mL Falcon tube.
- Add 10 mL distilled water and one drop 1-octanol.
- Shake and incubate for 15 minutes in a boiling water bath; cool down to room temperature and transfer to a 25 mL volumetric flask.
- Add 1 mL concentrated Carrez I solution, shake, add 1 mL concentrated Carrez II solution, shake, and dilute to the mark with 0.1 M NaOH.
- Shake and filter through a fluted paper filter.
- Use 1000 µL sample solution for analysis.

4. Manual test procedure

Wavelength:	340 nm
Temperature (measurement):	20 – 37 °C (68 – 99 °F)
Photometer alignment:	against air (without cuvette)
Measuring range:	10 – 600 mg/L (for 100 µL)

	Reagent blank	**Samples / controls**
Reagent 1	2000 µL	2000 µL
Sample / control	-	100 µL
Dist. water	100 µL	-
Mix, incubate for **3 minutes at 20 – 37 °C (68 – 99 °F)**. **Read absorbance A_1**, then addition of:		
Reagent 2	500 µL	500 µL
Mix, incubate for **15 minutes at 20 – 37 °C (68 – 99 °F)** and **read absorbance A_2**.		

4.1. Important notes for assay procedure

- **Please note:** incubation and measurement at **37 °C (99 °F)** requires limiting the upper measurement range to **400°mg/L** (dilute samples into this range if necessary) **or** calibration, as with automated testing.
- The reagent blank value (water sample) must be determined **in each series of measurement** and subtracted from **each** sample result.
- Specified incubation times were validated at 25 °C (77 °F). The test may generally perform within a range between **20 – 37 °C (68 – 99 °F)**.
- Use separate tips for each sample extract and the control solutions to avoid cross-contamination; rinse the tip before pipetting.
- A multistepper pipette is recommended for adding reagents. Use a separate tip for each component.
- Stirring spatulas are recommended for mixing each individual cuvette. Remove these from the cuvette immediately before measuring the absorbance
- Always wait for the reaction to end or for the absorbance to stabilize (at least during the first test runs or validation). If the absorbance has not stopped after the recommended incubation time, continue measuring at 5-minute intervals, for example, until a constant absorbance value is reached.
- If a creep reaction occurs, the reaction will not have finished after stated incubation times and will typically show a constant increase of ΔA. Calculate the analyte-specific ΔA value by plotting the absorbance values against time and performing a linear regression to determine the rate of increase in ΔA per minute related to the creep reaction. Then, extrapolate the absorbance to the time at which reagent 2 is added.
- If the measured absorbance difference of the samples is too small (< 0.020), the sample solution must be prepared again with a higher weight or a lower dilution.
- If the absorbance difference of the samples is very large (e.g., > 1.500), the sample solution must be diluted if necessary.

5. Calculation of results

5.1. Calculation of sample solutions

5.1.1. Concentration of L-lactic acid

The extinction difference ΔA must be calculated for each sample:

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

df: Dilution factor
RB: Reagent blank

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

The specified df value of **0.808 applies to a base application of 100 µL**. An increase in sample volume is possible (max. 1000 µL; refer to validation report). **While keeping reagent volumes unchanged**, this requires **conversion of the reagent dilution factor (df)** accordingly.

Increasing the sample volume may influence test performance. This must generally be checked depending on the matrix. **The reagent blank value must be adjusted to the changed sample volume.**

The concentration of L-Lactic acid is calculated using Lambert-Beer's law:

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

If the sample solution was diluted before measurement, this result has to be multiplied with the **sample pre-dilution factor F**.

V:	Test volume basic application [mL]	= 2.600
MW:	Molecular weight L-lactic acid [g/mol]	= 90.1
d:	Optical path [cm]	= 1.00
v:	Sample volume [mL]	= 0.100
ε:	Extinction coefficient NADH [L/mmol × cm]	= 6.3 (at 340 nm)

5.2. Calculation of solid samples

When analyzing solid and semi-solid samples that have to be weighed in for the extraction of the sample, the content is related to the sample weight:

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

5.3. Controls & acceptance criteria

Control or reference samples should be included in each run for quality control purposes. Therefore, we recommend Enzytec™ Liquid Multi-Acid Standard low (Art. No. E8460; 0.250 g/L L-lactic acid).

The recovery of this multi-standard low and other aqueous control solutions should be 100 ± 5 %.

As a certified (standard) reference material, we recommend:

- *Standardwein der Deutschen Weinanalytiker* (Standard wine of the German Wine Analysts); Label “hellblau”; https://www.weinanalytiker.de/standard-testloesung/

6. Performance data

6.1. Specificity & side activities

The test is specific to L-lactic acid. Several organic acids were tested with up to 100 g/L for a potential side-activity in absence of L-lactic acid (applied sample volume 100 µL): D-lactic acid (100 and 10 g/L), pyruvic acid (3, 1, and 0.5 g/L), D-/L-malic acid (40 and 20 g/L), acetic acid, butyric acid, citric acid, formic acid, oxalic acid, propionic acid, and tartaric acid (20 g/L each). None of these substances showed side-activities at these concentrations.

Ascorbic acid, 3-hydroxybutyric acid, and sulphite were found to have a low activity, but only at concentrations higher than 0.2, 0.05, and 0.1 g/L, respectively.

6.2. Interferences

An interference study was conducted in the presence of 0.1 or 0.2 g/L L-lactic acid. The tested substances included pyruvate (3.0, 1.0, and 0.5 g/L), formic acid, D-/L-malic acid, butyric acid, citric acid, acetic acid, oxalic acid, propionic acid, L-tartaric acid, sorbic acid (each 20 g/L), oxaloacetic acid (20, 2, and 0.2 g/L), ethanol (100, 30, and 10 g/L), glucose, sucrose, lactose, and galactose (each 100 g/L), glycerol and sorbitol (each 10 g/L), and fructose (100, 75, 50, 25, 20, 15, 12.5, 10, and 5 g/L). Pyruvate showed interference only at 3.0 g/L, while fructose does interfere above 10 g/L. No relevant interference was observed for the remaining tested substances.

In contrast, 3-hydroxybutyric acid, ascorbic acid, and SO_2 interfere in the presence of 0.1 g/L L-lactic acid at concentrations of 0.05 g/L, 0.2 g/L, and 0.05 g/L respectively.

6.3. Linearity, measuring range & sensitivity

Linearity is given up to 600 mg/L L-lactic acid (sample volume of 100 µL) with a recommended measuring range of 10 – 600 mg/L.

The limit of detection (LoD) was determined according to the DIN 32645:2008-11 method in a buffered aqueous solution. For a sample volume of 100 µL, the calculated LoD is 4.0 mg/L.

The limit of quantification (LoQ) was determined by precision profile. The calculated LoQ is 10.0 mg/L for a sample volume of 100 µL.

The smallest absorbance difference that the method can distinguish is ΔA = 0.005. For a sample volume of v = 1000 µL, this results in an LoD of 0.25 mg/L. Based on ΔA = 0.010, an LoQ of 0.5 mg/L was calculated.

6.4. Automation with Pictus 500

6.4.1. Limit of quantification (LoQ)

P500 application	LoQ
High Range	40 mg/L
Basic Range	15 mg/L
Sensitive Range	0.75 mg/L

6.4.2. Measuring ranges

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

6.4.3. Precision and accuracy

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

High Range

Target concentration, mg/L	150	450
Mean value, mg/L	151.8	459.3
SD, mg/L	1.77	4.06
RSD, %	1.17	0.88
Recovery, %	101.2	102.1

Basic Range

Target concentration, mg/L	150	450
Mean value, mg/L	151.4	459.4
SD, mg/L	0.93	2.36
RSD, %	0.61	0.51
Recovery, %	100.9	102.1

Sensitive Range

Target concentration, mg/L	15	45
Mean value, mg/L	15.38	44.87
SD, mg/L	0.13	0.27
RSD, %	0.87	0.59
Recovery, %	102.5	99.7

7. Supporting documents

On request, we offer the following documents:

- Enzytec™ *Liquid* L-Lactic acid Validation Report
- Enzytec™ *Liquid* Sample preparation guide
- Enzytec™ *Liquid* L-Lactic Excel template for results
- Enzytec™ *Liquid* L-Lactic Technical information
- Enzytec™ *Liquid* Troubleshooting guide

Safety data sheets (SDS) and certificates of analysis (CoA) are available in digital form, quoting the batch number, via the following link:

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

8. Limits of this method

Test results may vary depending on the sample matrix, specific test implementation, and laboratory environmental conditions. Detection and quantification limits are dependent on respective sample matrices extraction procedures. Refer toto the current Validation Report for details.

For this test, only the matrices explicitly listed in the documentation were validated, due to the wide variety of food products and other potential sample materials.

When analysing non-validated matrices results should be verified by performing spiking (fortification) experiments. If appropriate or necessary, a suitable sample preparation procedure for the respective matrix must be developed and validated.

The responsibility for validating non-validated matrices and for ensuring the suitability of the assay for its intended use lies solely with the user.

9. Services & technical support

Upon request, we offer the following services, among others:

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

10. Disclaimer

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b. Failure to use trained and qualified personnel;
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