

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 (36 – 46 °F)

This test was evaluated using selected samples of the following matrices: milk and (fermented) milk products, fermented vegetable products, wines, beer, fruit and vegetable juices, and egg 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* 2024.06 First Action. A publication is available in J. AOAC Int. 108(5), 708–723 (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

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.

## 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 / 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

### 3.1. Content & composition

- 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).
- Red wines need to be diluted only (typically 1:5 with water); use 100 µL sample volume for analysis.
- 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 polyvinylpyrrolidone (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  $\text{K}_4[\text{Fe}(\text{CN})_6] \times 3 \text{H}_2\text{O}/100 \text{ mL}$ ), 5 mL Carrez II solution (7.20 g zinc sulfate  $\text{ZnSO}_4 \times 7 \text{H}_2\text{O}/100 \text{ mL}$ ) 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).
- Concentrated Carrez reagents (see sections 3.2.5. – 3.2.6.):
  - Carrez I solution: 15 g  $\text{K}_4[\text{Fe}(\text{CN})_6] \times 3 \text{H}_2\text{O}/100 \text{ mL}$
  - Carrez II solution: 30 g  $\text{ZnSO}_4 \times 7 \text{H}_2\text{O}/100 \text{ mL}$
- 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.

### 3.2. Representative Application Examples consistent with published AOAC methods and §64 52.01.01-5 LFGB

**Note:** The recommendations for sample preparation described below are in accordance with the published AOAC method. Alternatives may be used if properly validated and verified by the user.

#### 3.2.1. Sausages

- 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 MKOH 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.

**3.2.2. 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.
- Dilute to 100 mL with distilled water; filter through a fluted paper filter or centrifuge at 3000 g for at least 5 minutes until a clear supernatant is obtained.
- Use 100 µL sample solution for analysis.

**3.2.3. 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 g for at least 5 minutes until a clear supernatant is obtained.
- Use 1000 µL sample solution for analysis.

**3.2.4. 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 g for at least 5 minutes until a clear supernatant is obtained.
- Use 1000 µL sample solution for analysis.

**3.2.5. 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.

**3.2.6. 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. Assays performance**

Wavelength: 340 nm  
 Temperature (measurement): 20 – 37 °C (68 – 99 °F)  
 Photometer alignment: against air (without cuvette)  
 Measuring range: 15 – 500 mg/L (for 100 µL)

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

**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 and established 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 multistep 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 D-lactic acid**

The extinction difference ΔA must be calculated for each sample:

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

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 D-Lactic acid is calculated using Lambert-Beer's law:

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

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 D-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:

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

### 5.3. Controls & acceptance criteria

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; with 0.250 g/L D-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); e.g. Label "hellblau"; <https://www.weinanalytiker.de/standard-testloesung/>

## 6. Performance data

### 6.1. Specificity & side activities

The test is specific for D-lactic acid. Relevant organic acids were tested for a potential side activity at several concentrations ranging from 0.2 to 100 g/L in absence of D-lactic acid: L-lactic acid, pyruvic acid, formic acid, D-/L-malic acid, butyric acid, citric acid, acetic acid, oxalic acid, propionic acid, L-tartaric acid, oxaloacetic acid and ascorbic acid. None of these substances showed significant side activities at these concentrations.

In contrast, 3-hydroxybutyric acid and sulfite were analyzed in more detail and showed a low activity at concentrations higher than 0.2, and 0.1 g/L, respectively.

### 6.2. Interferences

The following substances were tested in the presence of 0.2 g/L D-lactic acid: pyruvic acid, formic acid, D-/L-malic acid, butyric acid, citric acid, acetic acid, oxalic acid, propionic acid, L-tartaric acid, sorbic acid, oxaloacetic acid and ethanol. Pyruvic acid and oxaloacetic acid did not show interference up to 1 and 2 g/L. No other tested substances showed interferences at a concentration of 20 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.

Glucose, sucrose, lactose, and galactose do not interfere at concentrations of 100 g/L. Sugar alcohols, like glycerol and sorbitol do not interfere at concentrations of 10 g/L. In contrast, fructose does interfere above 10 g/L.

### 6.3. Linearity, measuring range & sensitivity

Linearity is given up to 500 mg/L D-lactic acid (sample volume of 100 µL) with a recommended measuring range of 15 – 500 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 5 mg/L.

The limit of quantification (LoQ) was determined by precision profile. The calculated LoQ is 15 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	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 D-Lactic acid Validation Report
- Enzytec™ Liquid Sample preparation guide
- Enzytec™ Liquid D-Lactic Excel template for results
- Enzytec™ Liquid D-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 to 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

On request, we offer the following services:

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

## 10. Disclaimer

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- c. Failure to apply appropriate industry standard practices, including Good Laboratory Practices;
- d. Failure to otherwise use, and when necessary validate or verify, suitable controls, samples, matrices, or processing procedures;
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