D-LACTIC ACID
(LIQUID READY™)

PRODUCT INSTRUCTIONS

SKU: 700007709
K-DATELQ

08/25

(50 Manual Assays per Kit) or
(500 Auto-Analyzer Assays per Kit)

Megazyme® by NEOGEN®

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INTRODUCTION:

D-Lactic Acid is found in many foods and beverages. Produced naturally by lactic acid bacteria, D-Lactic Acid is found in fermented milk products such as yogurt and cheese, and in pickled vegetables, cured meats and fish. The quality of milk, meat and fruit juice can be established by measurement of the D-Lactic Acid content. In the wine industry, the production of D-Lactic Acid can indicate wine spoilage by lactic acid bacteria.

PRINCIPLE:

The quantification of D-Lactic Acid requires two reactions. In the first reaction, catalyzed by D-lactate dehydrogenase (D-LDH), D-Lactic Acid (D-lactate) is oxidized to pyruvate in the presence of nicotinamide-adenine dinucleotide (NAD^+).

$$D-Lactate + NAD^+ \xrightleftharpoons{D\text{-}LDH} Pyruvate + NADH + H^+$$

Since the equilibrium of the reaction lies firmly in favor of the D-lactic acid and NAD^+, a further reaction is required to "trap" the pyruvate product. This is chemically catalyzed. The amount of NADH formed in the coupled reaction is stoichiometric to the amount of D-lactic Acid. The NADH is measured by the increase in absorbance at 340 nm.

SPECIFICITY, SENSITIVITY AND LINEARITY:

- The assay is specific to D-Lactic Acid.
- The limit of detection (LOD) is 0.004 g/L, and the limit of quantification (LOQ) is 0.011 g/L (using a sample volume of 0.1 mL).
- The recommended measuring range is between 0.02 and 0.5 g/L (using a sample volume of 0.1 mL). This corresponds to 2 - 50 µg of D-Lactic Acid per assay.

INTERFERENCE:

D-Fructose interferes at concentrations above 50 g/L. Ascorbic acid interferes at concentrations above 1 g/L and sulphites interfere at concentrations above 0.1 g/L. It is recommended that samples with stated contents of these interfering agents are diluted prior to testing.

SAFETY:

The general safety measures that apply to all chemical substances should be adhered to. After use, the reagents may be disposed of with standard laboratory waste, in accordance with local regulations and guidelines.

NOTE: For more information regarding the performance of this product please refer to the associated validation report available from the Megazyme website. For more information regarding the safe usage and handling of this product please refer to the associated SDS that is available from the Megazyme website.

KIT CONTENTS:

Kits are designed for use in both manual and automated workflows. The reagents are sufficient for performing 50 assays in manual format or 500 assays in auto-analyzer format. The kit contains:

Reagent 1 (2 x 50 mL): Buffer, D-LDH
Contains sodium azide (0.05% *w/v*) as a preservative. Ready to use.
Store at 4°C. See individual labels for expiry date.

Reagent 2 (2 x 12.5 mL): NAD^+
Ready to use.
Store at 4°C. See individual labels for expiry date.

Standard (5 mL): D-Lactic Acid standard (0.5 g/L).
Contains sodium azide (0.05% *w/v*) as a preservative. Ready to use.
Store at 4°C. See individual labels for expiry date.

NOTE: The D-Lactic Acid standard solution is only assayed where there is some doubt about the accuracy of the spectrophotometer being used or where it is suspected that inhibition is being caused by substances in the sample. The concentration of D-Lactic Acid is determined directly from the extinction coefficient of NADH.

PREPARATION OF REAGENT SOLUTIONS:

Bring all reagents to room temperature (20 - 25 °C) before use.

MANUAL ASSAY PROCEDURE:

Wavelength: 340 nm
Cuvette: 1 cm light path (glass or plastic)
Temperature: 20 - 37°C
Final volume: 2.60 mL
Sample solution: 0.02 g/L to 0.5 g/L (i.e. 2 - 50 µg of D-Lactic Acid per cuvette)
Read against air (without a cuvette in the light path) or against water

Pipette into Cuvettes	Blank	Sample
Reagent 1	2.0 mL	2.0 mL
Sample	-	0.1 mL
Distilled Water	0.1 mL	-
Mix*, incubate for ~ 3 minutes at 20 - 37°C, then read the absorbances (A_1) Add Reagent 2 as described below:		
Reagent 2	0.5 mL	0.5 mL
Mix*, incubate for ~ 10 minutes at 20 -37°C, then read the absorbances (A_2). **		

* Either by aspiration with the pipette tip used to dispense the liquid or by gentle inversion after sealing the cuvette with a cuvette cap or Parafilm®.
** It may be necessary to check if the reaction has reached completion by continuing to read the absorbances at 1 minute intervals. If the reaction has not reached completion continue to measure absorbances until the values measured either remain the same, or increase constantly over 1 minute. If this "creep" rate is greater for the sample than for the blank, extrapolate the absorbances (sample and blank) back to the time of addition of Reagent 2.

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

CALCULATION:

NOTE: These calculations can be simplified by using the *MegaCalc*™ tool, downloadable from the product page.

1. Calculation of the dilution factor (df)

Determine the dilution factor (df) based on the component ratios:

$$df = \frac{\text{Sample volume [mL] + R1 volume [mL]}}{\text{Total reaction volume [mL]}}$$

It follows for the D-Lactic Acid manual assay procedure:

$$df = \frac{0.1 + 2.0}{2.6} = 0.808$$

2. Calculation of the absorbance difference $\Delta A_{\text{D-Lactic Acid}}$

$$\Delta A_{\text{D-Lactic Acid}} = (A_2 - df \times A_1)_{\text{sample}} - (A_2 - df \times A_1)_{\text{blank}}$$

It follows for the D-Lactic Acid manual assay procedure:

$$\Delta A_{\text{D-Lactic Acid}} = (A_2 - 0.808 \times A_1)_{\text{sample}} - (A_2 - 0.808 \times A_1)_{\text{blank}}$$

NOTE: Increasing or decreasing the sample volume with unchanged reagent volumes requires recalculation of the dilution factor; if volumes are changed, the system and performance may be affected.

3. Calculation of the D-Lactic Acid content

The concentration of D-Lactic Acid can be calculated as follows:

$$c = \frac{V \times MW}{\varepsilon \times d \times v} \times \Delta A_{\text{D-Lactic acid}} \quad [g/L]$$

where:

V = final volume [mL]
MW = molecular weight of D-Lactic Acid [g/mol]
ε = extinction coefficient of NADH at 340 nm [l x mol^{-1} x cm^{-1}]
d = light path [cm]
v = sample volume [mL]

It follows for the D-Lactic Acid manual assay procedure:

$$c = \frac{2.6 \times 90.1}{6300 \times 1.0 \times 0.1} \times \Delta A_{\text{D-Lactic acid}} \quad [g/L]$$

$$= 0.3718 \times \Delta A_{\text{D-Lactic Acid}} \quad [g/L]$$

If the sample has been diluted during preparation, the result must be multiplied by the sample dilution factor, F.

4. Calculation of the D-Lactic Acid content in solid or semi-solid samples:

When analyzing solid and semi-solid samples which are weighed out for sample preparation, the content (g/100 g) is calculated from the amount weighed as follows:

$$\frac{c_{\text{D-Lactic acid}}\ [g/L \text{ sample solution}]}{\text{weight}_{\text{sample}}\ [g/L \text{ sample solution}]} \times 100 \quad [g/100g]$$

AUTO-ANALYZER ASSAY PROCEDURE:

This kit has been designed for auto-analyzers and can be adapted to most instruments. A sample method is shown below (validated on the Awareness ChemWell®-T analyzer).

NOTE: For each batch of samples that is applied to the determination of D-Lactic Acid a calibration curve must be performed concurrently using the same batch of reagents.

Parameter	Details
Wavelength	340/405 nm (primary/secondary)
Temperature	20 - 37°C
Test	End-point test with following test sequence: – Add Reagent 1 **[0.2 mL]** – Add Sample or Calibrator **[0.01 mL]** – Pre-incubate 1-3 minutes [20 - 37°C] – Measure A_1 at 340/405 nm – Add Reagent 2 **[0.05 mL]** – Incubate 10 minutes at [20 - 37°C] – Measure A_2 at 340/405 nm – Calculate $A_2 - A_1$ against calibration curve
Calibration	Calibrate using 2 – 4 calibrators ranging from 0 – 0.5 g/L. The calibration curve is linear. An example of how to use the standard supplied with the kit to create a calibration curve is shown below: Calibrator 1 0 g/L (use distilled water) Calibrator 2 0.05 g/L (dilute Standard 10-fold) Calibrator 3 0.25 g/L (dilute Standard 2-fold) Calibrator 4 0.5 g/L (use Standard as-is) *Perform all dilutions with distilled water.*

SAMPLE PREPARATION:

1. Sample dilution

The amount of D-Lactic Acid present in the sample should range from 0.02 g/L to 0.5 g/L. If the value of $\Delta A_{D\text{-}Lactic\ Acid}$ is too low (e.g. <0.1), weigh more sample or decrease the dilution. If the value $\Delta A_{D\text{-}Lactic\ Acid}$ is too high (e.g. >2.0), increase the dilution in distilled water.

Sample Dilution Table

Estimated Concentration of D-Lactic Acid (g/L)	Dilution with Water	Dilution factor (F)
< 0.5	No dilution required	1
0.5 – 5	1 mL sample + 9 mL water	10
5 - 50	1 mL sample + 99 mL water	100

2. General sample preparation guide

- Clear, slightly colored, and approximately neutral, liquid samples at a concentration up to 0.5 g/L D-Lactic Acid can be used directly in the assay.
- Turbid samples should be filtered or centrifuged.
- Acidic samples (pH < 3.0) must be neutralized to approximately pH 8.0.
- Samples containing carbon dioxide should be degassed by gentle agitation or stirring with a glass rod.
- Solid samples should be homogenized, extracted in water, and filtered or centrifuged if necessary.
- Strongly colored samples should be treated by the addition of 0.2 g of polyvinylpolypyrrolidone (PVPP) per 10 mL of sample in a tube. Shake the tube vigorously for 5 minutes and then filter through filter paper.
- Deproteinize samples using the Carrez Clarification Kit (700004270, K-CARREZ).
- Remove fat using the Carrez Clarification Kit (700004270, K-CARREZ).

3. Suggested sample preparation examples

(a) Determination of D-Lactic Acid in wine. Pass through a 0.2 micron syringe filter to clarify. Alternatively, centrifuge an aliquot of wine for 5 minutes at 15,000 g. *Typically, a 5-fold dilution in distilled water is required for red wine and no dilution is required for white wine.*

(b) Determination of D-Lactic Acid in beer (e.g. lager). Remove carbonation by stirring a sample in a beaker for approximately 60 seconds using a glass rod. Pass through a 0.2 micron syringe filter and use the clear filtrate in the assay. *Typically, no dilution is required.*

(c) Determination of D-Lactic Acid in fermented milk products (e.g. sour cream). Weigh 10 g of fermented product into a 50 mL volumetric flask, add the following solutions, and mix after each addition: 5 mL of Carrez I solution, 5 mL of Carrez II solution and 10 mL of NaOH solution (100 mM). Fill up to the mark

with distilled water, mix thoroughly and filter using a paper filter. *Typically, no further dilution is required.*

(d) Determination of D-Lactic Acid in meat products (e.g. salami). Homogenize solid samples in a blender and accurately weigh 5 g of representative material into a 100 mL vessel (e.g. Duran® bottle). Add 20 mL of 1 M perchloric acid and mix with a spatula for 5 minutes, or until the sample is evenly dispersed. Add 40 mL of distilled water and stir for another 10 minutes using a stir bar. Adjust the pH to 7.0 using 8 M potassium hydroxide, quantitatively transfer the suspension into a volumetric flask and make to 100 mL with distilled water. Store on ice or in a refrigerator for 20 minutes to precipitate potassium perchlorate and allow separation of the fat. Filter and discard the first 3-5 mL, and use the clear filtrate for the assay. *Typically, no further dilution is required.*

(e) Determination of D-Lactic Acid in sauerkraut juice. Pass through a 0.2 micron syringe filter to clarify. Alternatively, centrifuge an aliquot of juice for 5 minutes at 15,000 g. *Typically, a 20-fold dilution in distilled water is required.*

IMPORTANT NOTE: The above are suggested sample preparation examples only. If you have questions about these or other matrices, please contact your local sales representative for support.

SERVICES AND TECHNICAL SUPPORT

Please reach out to your local sales representative should you require any assistance, particularly in relation to:

- Troubleshooting
- Data analysis
- Additional matrix testing
- Application support in relation to automated analyzers

Supporting documents can be found in the product page:

- Quick Reference Guide
- MegaCalc™
- Safety Data Sheets (SDS)
- Certificates Of Analysis (COA)
- Validation Report

Contact us for more information: neogen.com/contact

Without guarantee

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User Responsibility:

- Users are responsible for familiarizing themselves with product instructions and information. Visit our website at neogen.com or contact your local Neogen® representative or authorized distributor for more information.
- When selecting a test method, it is important to recognize that external factors such as sampling methods, testing protocols, sample preparation, handling, laboratory technique and the sample itself may influence results.
- It is the user's responsibility in selecting any test method or product to evaluate a sufficient number of samples with the appropriate matrices and challenges to satisfy the user that the chosen test method meets the user's criteria.
- It is also the user's responsibility to determine that any test methods and results meet its customers' and suppliers' requirements.
- As with any test method, results obtained do not constitute a guarantee of the quality of the matrices or processes tested.

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