# Micro D-Lactic acid (D-LA) Assay Kit

## Cat #: orb3148273 (manual)

### Product Features

**Product name:** Micro D-Lactic acid (D-LA) Assay Kit

**Catalog number:** orb3148273

**Sample Types:** Animal and Plant Tissues, Cells or Bacteria, Serum (Plasma)

**Detection range:** 0.031-1 μmol/mL

**Sensitivity:** 0.031 μmol/mL

**Storage:** Stored at -20℃ for 6 months, protected from light

### Assay Principle

Lactic acid is an important intermediate product in the metabolic process of organisms, which is closely related to glucose metabolism, lipid metabolism, protein metabolism and intracellular energy metabolism. The content of lactic acid is an important indicator to evaluate glycogen metabolism and aerobic metabolism. Abnormally high concentrations of lactic acid are associated with pathological conditions such as cancer, diabetes, and lactic acidosis. Among them, D-lactic acid (D-LA) can be oxidized by D-lactate dehydrogenase to produce products that interact with tetrazolium salt WST-8 dye, forming a colored product that is proportional to the concentration of D-lactic acid in the sample, and its maximum absorption peak is at 450 nm.

### Kit Components

| Kit components | Size (96 T) | Storage conditions |
|---|---|---|
| Extraction Buffer | 75 mL×2 | 4℃ |
| Reagent I | 1.4 mL | -20℃ |
| Reagent II | 1.2 mL | -20℃ |
| Reagent III | 700 μL | -20℃, protected from light |
| Reagent IV | 140 μL | -20℃, protected from light |
| Standard (100 μmol/mL) | 100 μL | -20℃ |

**Note: Before formal testing, it is recommended to select 2-3 samples with large expected differences for pre-experiment.**

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## Materials Required but Not Supplied

- Microplate reader or visible spectrophotometer capable of measuring absorbance at 450 nm
- 96-well plate or microglass cuvette, precision pipettes, disposable pipette tips
- Thermostatic incubator, ice maker, centrifuge
- Deionized water
- Homogenizer (for tissue samples)

## Reagent Preparation

**Extraction Buffer:** Ready to use as supplied. Equilibrate to room temperature before use. Store at 4°C.

**Reagent I:** Ready to use as supplied. During the whole experiment, it was placed on ice; The unused reagent shall be stored at -20°C after subpackaging, and repeated freezing and thawing are prohibited.

**Reagent II:** Ready to use as supplied. During the whole experiment, it was placed on ice; The unused reagent shall be stored at -20°C after subpackaging, and repeated freezing and thawing are prohibited.

**Reagent III:** Ready to use as supplied. During the whole experiment, it was placed away from light on ice; The unused reagents are subpacked and stored at -20°C away from light, and repeated freezing and thawing are prohibited.

**Reagent IV:** Ready to use as supplied. During the whole experiment, it was placed away from light on ice; The unused reagents are subpacked and stored at -20°C away from light, and repeated freezing and thawing are prohibited.

**Working Reagent:** Prepare 55 µL working reagent for each well, prepare and use it now: suck 31 µL Extraction Buffer, 8 µL Reagent II, 5 µL Reagent III, 1 µL Reagent IV and 10 µL Reagent I, mixing evenly.

**Standard (100 µmol/mL):** Take 10 µL Standard 990 µL Extraction Buffer diluted to 1 µmol/mL, mixed evenly; Equilibrate to room temperature; The unused Standard (100 µmol/mL) are subpacked and stored at -20°C.

**Standard preparation:** Use 1 µmol/mL standard, prepare standard curve dilution as described in the table.

| Num. | Standard Volume | Extraction Buffer Volume (µL) | Concentration (µmol/mL) |
|---|---|---|---|
| Std.1 | 400 µL 1 µmol/mL Standard | 0 | 1 |
| Std.2 | 200 µL of Std.1 (1 µmol/mL) | 200 | 0.5 |
| Std.3 | 200 µL of Std.2 (0.5 µmol/mL) | 200 | 0.25 |
| Std.4 | 200 µL of Std.3 (0.25 µmol/mL) | 200 | 0.125 |
| Std.5 | 200 µL of Std.4 (0.125 µmol/mL) | 200 | 0.063 |
| Std.6 | 200 µL of Std.5 (0.063 µmol/mL) | 200 | 0.031 |
| Blank | 0 | 200 | 0 |

**Notes: Always prepare fresh standards per use; Diluted Standard Solution is unstable and must be used within 4 h.**

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## Sample Preparation

**Note: We recommend that you use fresh samples. If not assayed immediately, samples can be stored at -80°C for one month.**

1. Animal and plant tissues: Weigh about 0.1 g of sample, add 1 mL of precooled Extraction Buffer, homogenize in ice bath, centrifuge at 12,000 g, 4°C for 5 min, take the supernatant, and put it on ice for testing.
2. Bacteria or cells: Collect 5million cells or bacteria into a centrifuge tube, wash the cells with cold PBS, discard the supernatant after centrifugation, add 1 mL Extraction Buffer, break the cells with ice bath ultrasonic for 5 min (power 20% or 200 W, ultrasonic for 3 s, interval 7 s, repeat 30 times), then centrifuge at 12,000 g, 4°C for 5 min, take the supernatant, and put it on ice for testing.
3. Serum (plasma): Direct detection.

**Note: If the protein concentration of the sample is need to determined, it is recommended to use biorbyt Cat: orb1147876 Protein Quantification Kit (BCA Assay) to measure the protein concentration of the sample.**

## Assay Procedure

1. Preheat the microplate reader or visible spectrophotometer for more than 30 min, and adjust the wavelength to 450 nm, visible spectrophotometer was returned to zero with deionized water.
2. Operation table (The following operations are operated in the 96-well plate or microglass cuvette):

| Reagent | Test Well (µL) | Standard Well (µL) | Blank Well (µL) |
|---|---|---|---|
| Sample | 50 | 0 | 0 |
| Standard | 0 | 50 | 0 |
| Extraction Buffer | 0 | 0 | 50 |
| Working Reagent | 50 | 50 | 50 |

3. After mixing, incubate at 37°C in the dark for 30 min, and measure the absorbance at 450 nm, which was recorded as $A_{Test}$, $A_{Standard}$, and $A_{Blank}$. Calculate $\Delta A_{Test}=A_{Test}-A_{Blank}$, $\Delta A_{Standard}=A_{Standard}-A_{Blank}$.

**Note: Blank well and standard well only need to be measured once. Before the experiment, it is recommended to select 2-3 samples with large expected differences for pre experiment. If $\Delta A_{Test}$ is less than 0.03, the sample size can be appropriately increased; If $\Delta A_{Test}$ is greater than 1.0, the sample can be further diluted with Extraction Buffer, and the calculated result is multiplied by the dilution factor, or the sample size for extraction can be reduced.**

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## Data Analysis

**Note: We provide you with calculation formulae, including the derivation process and final formula. The two are exactly equal. It is suggested that the concise calculation formula in bold is final formula.**

1. Drawing of standard curve:

With the concentration of the standard solution as the x-axis and the $\Delta A_{Standard}$ as the y-axis, draw the standard curve, get the standard equation, and bring the $\Delta A_{Test}$ into the equation to get the x value (μmol/mL).

2. Calculation of D-LA content:

(1) Calculated by fresh weight of samples

D-LA content(μmol/g fresh weight)=$x \times V_{Sample} \div (W \times V_{Sample} \div V_{Sample\ total}) \times n$=**$x \div W \times n$**

(2) Calculated by protein concentration

D-LA content(μmoL/mg prot)=$x \times V_{Sample} \div (V_{Sample} \times Cpr) \times n$=**$x \div Cpr \times n$**

(3) Calculated by volume of liquid samples

D-LA content(μmol/mL)=$x \times V_{Sample} \div V_{Sample} \times n$=**$x \times n$**

(4) Calculated by number of cells or bacteria

D-LA content(μmol/$10^4$)=$x \times V_{Sample} \div (500 \times V_{Sample} \div V_{Sample\ total}) \times n$=**$x \div 500 \times n$**

$V_{Sample}$: add sample volume, 0.05 mL; W: weight of sample, g; $V_{Sample\ total}$: add Extraction Buffer volume to sample, 1 mL; n: the sample dilution factor; Cpr: sample protein concentration, mg/mL; 500: Total number of cells or bacteria, $5 \times 10^6$.

## Typical Data

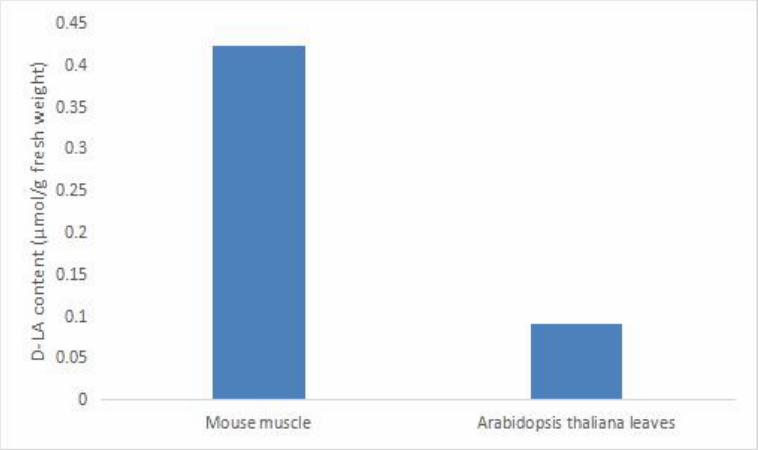


Figure 1. Determination D-LA content in Mouse muscles and Arabidopsis thaliana leaves by this assay kit

## Disclaimer

The reagent is only used in the field of scientific research, not suitable for clinical diagnosis or other purposes. For your safety and health, please wear a lab coat and disposable gloves.

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