



D-Lactate

Colorimetric Microplate Assay Kit

User Manual

Catalog # CAK1298

(Version 1.2A)

Detection and Quantification of D-Lactate content in Serum, Plasma,
Cell culture media, Other biological fluids Samples.

For research use only. Not for diagnostic or therapeutic procedures.

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I. INTRODUCTION

D-Lactate is the result of anaerobic glycolysis by microorganisms in the gastrointestinal system, and the product of detoxification of methylglyoxal by the glyoxalase system. The presence of abnormal levels of D-Lactate has been linked to a series of pathological conditions, such as diabetes, and appendicitis.

D-Lactate Microplate Colorimetric Assay Kit is a sensitive assay for determining D-lactate content in various samples. The kit is based on D-lactate dehydrogenase catalyzed oxidation of D-lactate, in which the formed NADH reduces a formazan reagent. The intensity of the product color, measured at 450nm, is proportionate to the D-lactate concentration in the sample.

II.KIT COMPONENTS

Component	Volume	Storage
96-Well Microplate	1 plate	
Assay Buffer	30 ml x 4	4 °C
Reaction Buffer	6 ml x 1	4 °C
Enzyme	Powder x 1	-20 °C
Coenzyme	Powder x 1	-20 °C
Dye ReagentA	Powder x 1	4 °C, keep in dark
Dye Reagent B	1 ml x 1	4 °C, keep in dark
Standard	20µl x 1	4 °C
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III. MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at 450 nm
2. Distilled water
3. Pipettor, multi-channel pipettor
4. Pipette tips
5. Centrifuge
6. Timer

IV. REAGENT PREPARATION

Standard: Briefly centrifuge prior to opening. Transfer 4.2 μ l into 1 ml distilled water to dissolve to generate 50 mmol/L of standard top solution, store at 4°C for 1 month or -20°C for 4-5 months after reconstitution. Perform 2-fold serial dilutions of the top standard solution using distilled water to make the standard curve. The concentration of standard curve could be 50/25/12.5/6.25/3.12/1.56/0.78 mmol/L.

Enzyme: Briefly centrifuge prior to opening. Add 1 ml Assay Buffer to dissolve before use. Prepare for immediate use or store at -80°C for 1 month.

Coenzyme: Briefly centrifuge prior to opening. Add 1 ml Assay Buffer to dissolve before use. Keep in dark and store at 4°C for 1 week or -20 °C for 2 months.

Dye Reagent A: Add 9 ml distilled water to dissolve before use, mix. Keep in dark and store at 4°C for 1 week or -20°C for 1 month.

Note: Divide into small aliquots to avoid repeated freeze-thaw cycles.

V. SAMPLE PREPARATION

1. For liquid samples

Detect directly, or dilute with Assay Buffer.

2. For cell and bacteria samples

Collect cell or bacteria into centrifuge tube, discard the supernatant after centrifugation, add 1 ml Assay Buffer for 5×10^6 cell or bacteria, sonicate (with power 20%, sonicate 3s, interval 10s, repeat 30 times); centrifuged at 10000g 4°C for 10 minutes, take the supernatant into a new centrifuge tube for detection.

3. For tissue samples

Weigh out 0.1 g tissue, homogenize with 1 ml Assay Buffer on ice, centrifuged at 10000g 4°C for 10 minutes, take the supernatant into a new centrifuge tube for detection.

VI. ASSAY PROCEDURE

Add following reagents into the microplate:

Reagent*	Sample**	Standard	Blank
Reaction Buffer	60 μ l	60 μ l	60 μ l
Sample	20 μ l	--	--
Standard	--	20 μ l	--
Distilled water	--	--	20 μ l
Enzyme	10 μ l	10 μ l	10 μ l
Coenzyme	10 μ l	10 μ l	10 μ l
Mix, keep at room temperature for 5 minutes.			
Dye Reagent A	90 μ l	90 μ l	90 μ l
Dye Reagent B	10 μ l	10 μ l	10 μ l
Mix, keep at room temperature for 20 minutes, record absorbance measured at 450nm.			

Note:

*Reagents must be added sequentially and should not be premixed prior to addition.

**The concentrations can vary over a wide range depending on the different samples.

For unknown samples, we recommend doing a pilot experiment & testing several doses to ensure the readings are within the standard curve range.

**For colored samples, we recommend setting a parallel sample background control well with same volume of sample only. Other reagents were replaced by distilled water to the same total volume. Subtract the OD value of the sample background control from the OD value of the sample to correct for interference from the sample's own color.

VII. CALCULATION

1. Calculate the sample concentration in ASSAY PROCEDURE according to the slope of the standard curve

$$C = \frac{(OD_{\text{Sample}} - OD_{\text{Blank}}) - \text{Intercept}}{\text{Slope}} \times n (\text{mmol/L})$$

Calculate the initial concentration according to sample preparation procedure.

2. According to one point of the standard OD and concentration

2.1 According to the quantity of cells or bacteria

$$C = \frac{(C_{\text{Standard}} \times V_{\text{Standard}}) \times (OD_{\text{Sample}} - OD_{\text{Blank}})}{(OD_{\text{Standard}} - OD_{\text{Blank}}) \times N \times (V_{\text{Sample}} / V_{\text{Assay}})} (\mu\text{mol}/10^4)$$

2.2 According to the weight of sample

$$C = \frac{(C_{\text{Standard}} \times V_{\text{Standard}}) \times (OD_{\text{Sample}} - OD_{\text{Blank}})}{(OD_{\text{Standard}} - OD_{\text{Blank}}) \times W \times (V_{\text{Sample}} / V_{\text{Assay}})} (\mu\text{mol/g})$$

2.3 According to the volume of sample

$$C = \frac{(C_{\text{Standard}} \times V_{\text{Standard}}) \times (OD_{\text{Sample}} - OD_{\text{Blank}})}{(OD_{\text{Standard}} - OD_{\text{Blank}}) \times V_{\text{Sample}}} (\mu\text{mol/ml})$$

Slope: the absorbance slope of standard curve

n: the dilution factor

C_{Standard} : the standard concentration, mmol/L = $\mu\text{mol/ml}$

V_{Standard} : the volume of standard in assay procedure, μl

V_{Sample} : the volume of sample in assay procedure, μl

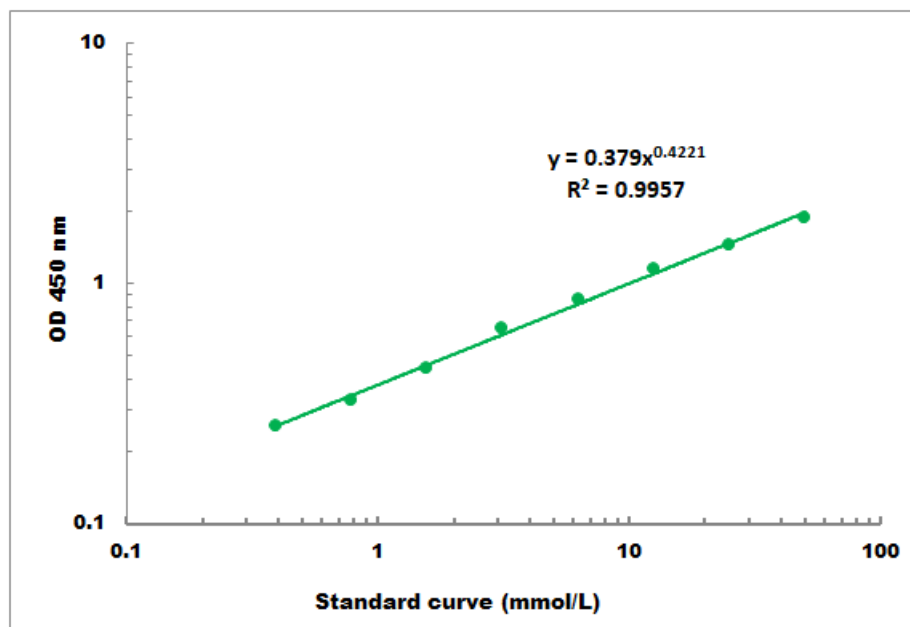
V_{Assay} : the volume of Assay Buffer, μl

W: the weight of sample, g

N: the quantity of cell or bacteria, 10^4

VIII. TYPICAL DATA

The standard curve is for demonstration only. A standard curve must be run with each assay.



Detection Range: 0.5 mmol/L - 50 mmol/L