D-Lactate Microplate Assay Kit

User Manual

Catalog # CAK1298

(Version 1.1A)

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.

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 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 450 nm, 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 Reagent A	Powder x 1	4 °C
Dye Reagent B	1 ml x 1	4 °C
Standard	5 µl x 1	4 °C
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Note:

Enzyme: add 1 ml Assay Buffer to dissolve before use, store at -80°C for 1 month.

Coenzyme: add 1 ml Assay Buffer to dissolve before use, store at -80°C for 1 month.

Dye Reagent A: add 9 ml distilled water to dissolve before use, mix, store at 4°C for 1 month.

Standard: add 1 ml distilled water to dissolve before use, the concentration will be 50 mmol/L, store at 4°C for 1 month.

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. 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.

V. ASSAY PROCEDURE

Add following reagents into the microplate:

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

Note:

1) Perform 2-fold serial dilutions of the top standards to make the standard curve.

2) 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.

3) Reagents must be added step by step, can not be mixed and added together.

VI. CALCULATION

1. According to the volume of sample

$$\text{D-Lactate (μmol/ml)} = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / V_{Sample}$$

$$= 50 \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank})$$

2. According to the weight of sample

$$\text{D-Lactate (μmol/g)} = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / (W \times V_{Sample} / V_{Assay})$$

$$= 50 \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / W$$

3. According to the quantity of cell or bacteria

$$\text{D-Lactate (μmol/10}^4\text{)} = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / (N \times V_{Sample} / V_{Assay})$$

$$= 50 \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / N$$

$C_{Standard}$: the standard concentration, 50 mmol/L = 50 μmol/ml;

W: the weight of sample, g;

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

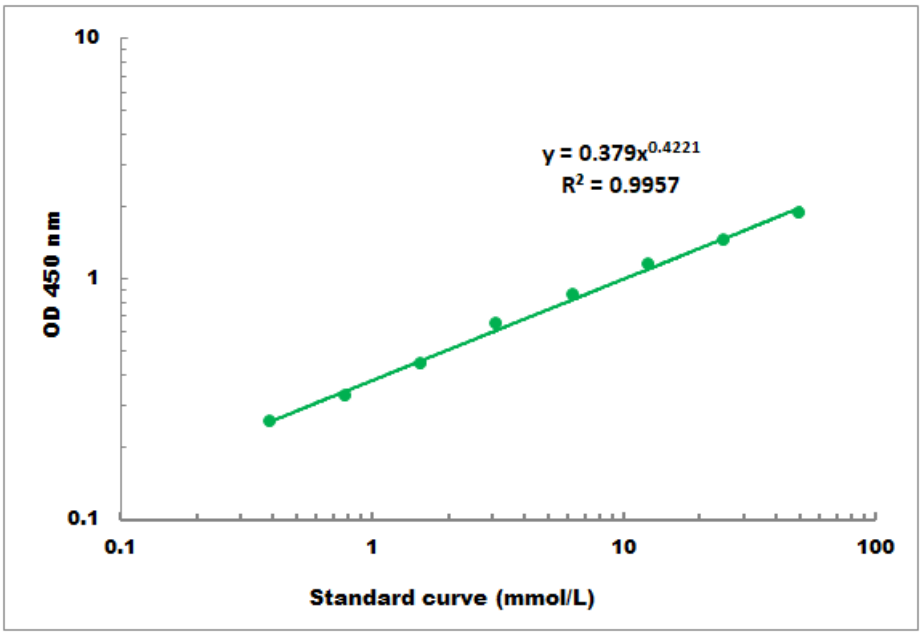
$V_{Standard}$: the volume of standard, 20 μl = 0.02 ml;

V_{Sample}: the volume of sample, 20 μl = 0.02 ml;

V_{Assay}: the volume of Assay buffer, 1 ml.

VII. 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

VIII. TECHNICAL SUPPORT

For troubleshooting, information or assistance, please go online to www.cohesionbio.com or contact us at techsupport@cohesionbio.com

IX. NOTES