Lactate Microplate Assay Kit

Cat #: orb707348 (manual)

Detection and Quantification of Lactate (LA) 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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INTRODUCTION

L-lactate is constantly produced from pyruvate via the enzyme lactate dehydrogenase (LDH) in a process of fermentation during normal metabolism and exercise. It does not increase in concentration until the rate of lactate production exceeds the rate of lactate removal, which is governed by a number of factors, including monocarboxylate transporters, concentration and isoform of LDH, and oxidative capacity of tissues. The concentration of blood lactate is usually 1-2 mM at rest, but can rise to over 20 mM during intense exertion and as high as 25 mM afterward. In addition to other biological roles, L-lactic acid is the primary endogenous agonist of hydroxycarboxylic acid receptor 1 (HCA1), which is a Gi/o-coupled G protein-coupled receptor (GPCR).

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

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	Powder x 1	4 °C
Technical Manual	1 Manual	

Note:

Enzyme: add 1 ml Assay Buffer to dissolve before use.

Coenzyme: add 1 ml Assay Buffer to dissolve before use.

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

Standard: add 1 ml distilled water to dissolve before use, the concentration will be 100 mmol/L.

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

SAMPLE PREPARATION

1. For liquid samples

Detect directly, or dilute with Assay Buffer.

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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 5 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.

CALCULATION

1. According to the volume of sample

Lactate (μmol/ml) = ($C_{Standard}$ × $V_{Standard}$) × (OD_{Sample} - OD_{Blank}) / ($OD_{Standard}$ - OD_{Blank}) / V_{Sample}

= 100 × (OD_{Sample} - OD_{Blank}) / ($OD_{Standard}$ - OD_{Blank})

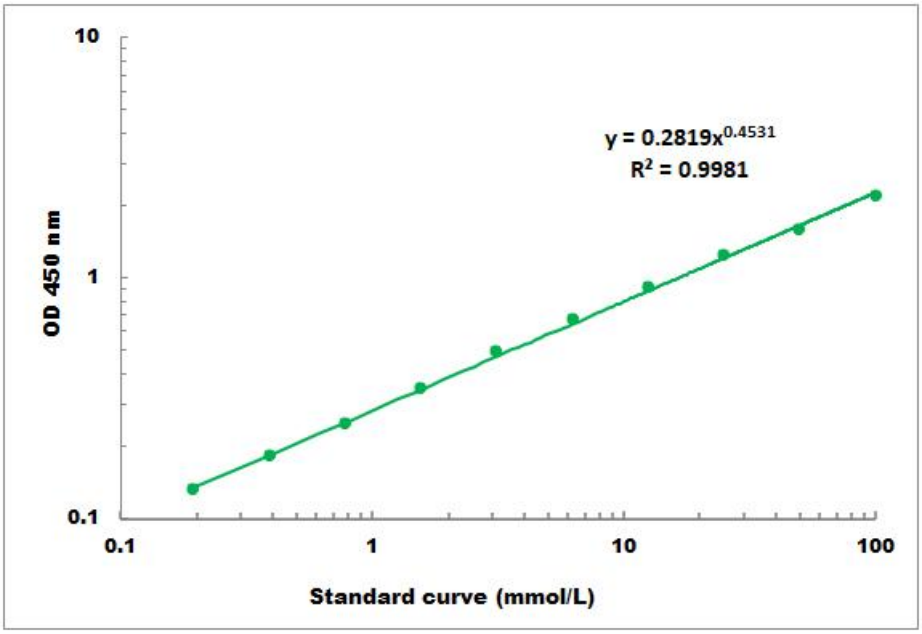
$C_{Standard}$: the standard concentration, 100 mmol/L = 100 μmol/ml;

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

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

TYPICAL DATA

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



Detection Range: 0.1 mmol/L - 100 mmol/L