



# **Lactate Microplate Assay Kit**

## **User Manual**

**Catalog # CAK1177**

(Version 1.4C)

Detection and Quantification of Lactate (LA) content in Serum,  
Plasma, Tissue extracts, Cell culture media, Other biological fluids  
Samples.

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

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## I. 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.

## 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	Powder x 1	4 °C
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### 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.

## 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 tissue samples

Weigh 0.1 g tissue, homogenize with 1 ml Assay buffer, centrifuged at 8000g 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 $\mu$ l	60 $\mu$ l	60 $\mu$ l
Sample	20 $\mu$ l	--	--
Standard	--	20 $\mu$ l	--
Distilled water	--	--	20 $\mu$ l
Enzyme	10 $\mu$ l	10 $\mu$ l	10 $\mu$ l
Coenzyme	10 $\mu$ l	10 $\mu$ l	10 $\mu$ l
Mix, keep at room temperature for 5 minutes.			
Dye Reagent A	90 $\mu$ l	90 $\mu$ l	90 $\mu$ l
Dye Reagent B	10 $\mu$ l	10 $\mu$ l	10 $\mu$ l
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.
- 3) Reagents must be added step by step, can not be mixed and added together.

## VI. CALCULATION

1. According to the volume of sample

$$\begin{aligned} \text{Lactate } (\mu\text{mol/ml}) &= (C_{\text{Standard}} \times V_{\text{Standard}}) \times (OD_{\text{Sample}} - OD_{\text{Blank}}) / (OD_{\text{Standard}} - OD_{\text{Blank}}) / \\ &V_{\text{Sample}} \\ &= 100 \times (OD_{\text{Sample}} - OD_{\text{Blank}}) / (OD_{\text{Standard}} - OD_{\text{Blank}}) \end{aligned}$$

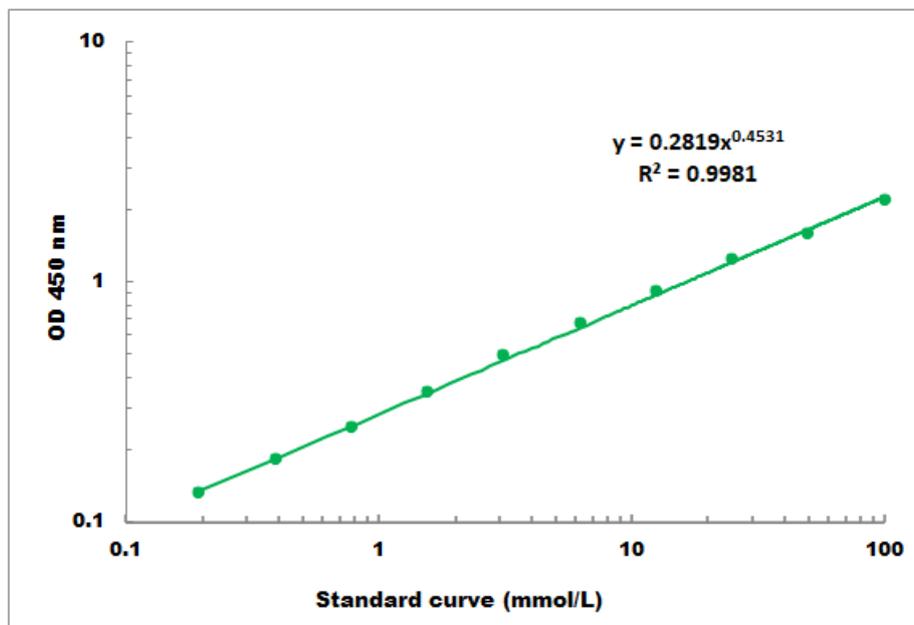
$C_{\text{Standard}}$ : the standard concentration, 100 mmol/L = 100  $\mu\text{mol/ml}$ ;

$V_{\text{Standard}}$ : the volume of standard, 20  $\mu\text{l}$  = 0.02 ml;

$V_{\text{Sample}}$ : the volume of sample, 20  $\mu\text{l}$  = 0.02 ml.

## VII. 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

## VIII. TECHNICAL SUPPORT

For troubleshooting, information or assistance, please go online to [www.cohesionbio.com](http://www.cohesionbio.com) or contact us at [techsupport@cohesionbio.com](mailto:techsupport@cohesionbio.com)

## IX. NOTES