Lactate Microplate Assay Kit

User Manual

Catalog # FTA0160

(Version 1.2A)

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.

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 (MTT) Reagent. The intensity of the product color, measured at 570 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	12 ml x 1	4 °C
Enzyme	Powder x 1	-20 °C
Coenzyme	Powder x 1	-20 °C
Dye Reagent	Powder 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: add 5 ml distilled water to dissolve before use.

Standard: add 1 ml distilled water to dissolve before use, the concentration will be 400 mmol/L.

III. MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at 570 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.

V. ASSAY PROCEDURE

Add following reagents into the microplate:

Reagent	Sample	Standard	Blank
Sample	20 µl	--	--
Standard	--	20 µl	
Distilled water	--	--	20 µl
Reaction Buffer	110 µl	110 µl	110 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	50 µl	50 µl	50 µl
Mix, keep at room temperature for 5 minutes, record absorbance measured at 570nm.			

Note:

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

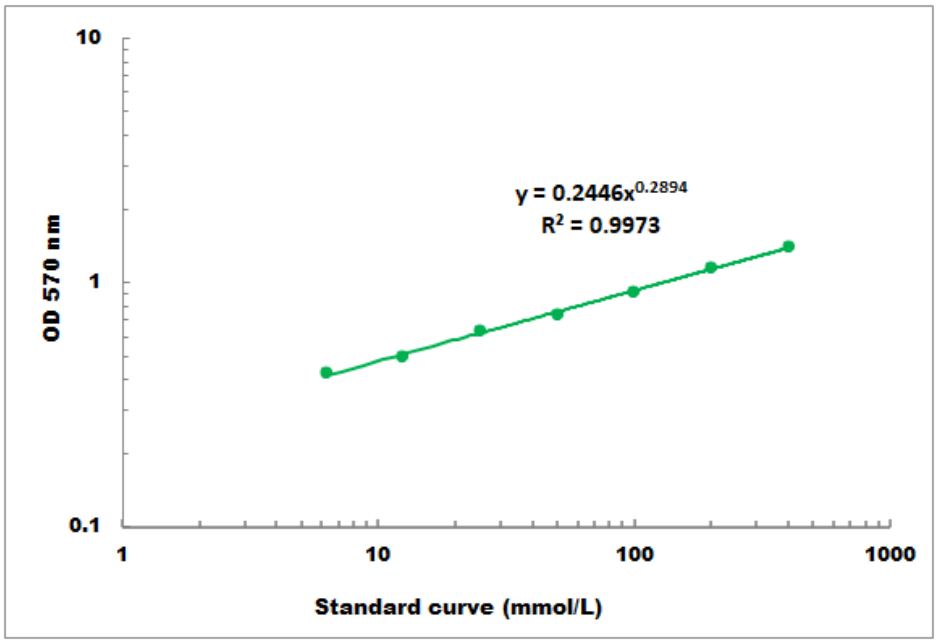
2) The concentrations can vary over a wide range depending on the different samples. For unknown samples, we recommend doing a pilot experiment & testing several doses to ensure the readings are within the standard curve range.

VI. CALCULATION

Subtract blank OD from the standard OD values and plot the OD against standard concentrations. Use the standard curve to determine the sample concentration.

VII. TYPICAL DATA

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



Detection Range: 4 mmol/L - 400 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