Lactate (D-Lactate) Assay Kit

Lactate (D-Lactate) Assay Kit is a detection kit for the quantification of Lactate (D-Lactate) in serum, plasma and cell culture media.

Catalog number: ARG82836

Package: 100 tests

TABLE OF CONTENTS

MANUFACTURED BY:

Arigo Biolaboratories Corporation

Address: No. 22, Ln. 227, Gongyuan Rd., Hsinchu City 300, Taiwan

Phone: +886 (3) 562 1738

Fax: +886 (3) 561 3008

Email: info@arigobio.com

INTRODUCTION

Lactic acid is an organic acid. It has a molecular formula $CH_3CH(OH)COOH$. It is white in the solid state and it is miscible with water. When in the dissolved state, it forms a colorless solution. Production includes both artificial synthesis as well as natural sources. Lactic acid is an alpha-hydroxy acid (AHA) due to the presence of a hydroxyl group adjacent to the carboxyl group. It is used as a synthetic intermediate in many organic synthesis industries and in various biochemical industries. The conjugate base of lactic acid is called lactate. [Provide by Wikipedia: Lactic acid]

PRINCIPLE OF THE ASSAY

This Lactate (D-Lactate) Assay Kit is a simple assay that measures the amount of lactate present in serum, plasma and cell culture supernatant. This assay 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 O.D. 565 nm, is proportionate to the lactate concentration in the sample.

MATERIALS PROVIDED & STORAGE INFORMATION

The kit is shipped on ice. Store all components at -20°C. Shelf life of six months after receipt.

Component	Quantity	Storage information
Assay Buffer	10 mL	-20°C
NAD Solution	1 mL	-20°C
MTT Solution	1.5 mL	-20°C
Enzyme A	120 µL	-20°C
Enzyme B	120 µL	-20°C
Standard (20 mM D-lactate)	1 mL	-20°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of reading at O.D. 565 nm
- Centrifuge and centrifuge tube
- Clear flat-bottom 96 well plate
- Deionized or distilled water
- Pipettes, pipette tips and Multichannel micropipette reservoir

TECHNICAL NOTES AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Reagents are for research use only. Normal precautions for laboratory reagents should be exercised while using the reagents. Please refer to Material Safety Data Sheet for detailed information.
- The following substances interfere and should be avoided in sample preparation: EDTA (>0.5 mM), ascorbic acid, SDS (>0.2%), sodium azide, NP-40 (>1%) and Tween-20 (>1%).
- This assay is based on an enzyme-catalyzed kinetic reaction. Addition of Working Reagent should be quick and mixing should be brief but thorough. Use of a multi-channel pipettor is recommended.
- All reagents should be mixed by gentle inversion or swirling prior to use. Do not induce foaming.
- Before using the kit, spin tubes and bring down all components to the bottom of tubes.
- It is highly recommended assaying the Standards and samples in duplicates.
- Change pipette tips between the addition of different reagent or samples.

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Serum: Collect blood in a tube with no anticoagulant. Allow the blood to clot at room temperature for 30 minutes. Centrifuge at 2500 x g for 20 minutes at 4°C.

Plasma: Collect blood with EDTA, heparin or citrate and centrifuge at 2000 x g for 10 minutes at 4°C.

Note:

- Serum and Plasma should be diluted at least 2× with distilled water prior to assay.
- For samples with potential endogenous enzyme activity (E.g., serum, plasma, tissue extracts), two reactions should be run: one with added Enzyme A and a No Enzyme A control (Blank).
- If the sample OD value is higher than OD for 2 mM D-lactate standard, dilute sample in distilled water and repeat the assay. Multiply the results by the dilution factor.

REAGENT PREPARATION

Working Reagent: for each reaction, mixing 60 µL of Assay Buffer, 1 µL of Enzyme A, 1 µL of Enzyme B, 10 µL of NAD Solution and 14 µL of MTT Solution. Fresh reconstitution is recommended.

Blank Working Reagent: for each reaction, mixing 60 µL of Assay Buffer, 1 µL of Enzyme B, 10 µL of NAD Solution and 14 µL of MTT Solution. Fresh reconstitution is recommended.

Standards: Prepare 1000 µL of 2.0 mM D-lactate Premix by mixing 100 µL of 20 mM Standard and 900 µL of distilled water. For cell culture samples containing phenol red, prepare 1000 µL of 1.0 mM lactate Premix by mixing 50 µL of 20 mM Standard and 950 µL of culture medium without serum. Dilute standard as follows.

Standard tube	D-lactate (mM)	Distilled water (µL)	Standard Premix (µL)
S1	2.0 or 1.0	0	100
S2	1.6 or 0.8	20	80
S3	1.2 or 0.6	40	60
S4	0.8 or 0.4	60	40
S5	0.6 or 0.3	70	30
S6	0.4 or 0.2	80	20
S7	0.2 or 0.1	90	10
S0	0	100	0

ASSAY PROCEDURE

Equilibrate all components to room temperature. Briefly centrifuge all tubes prior to use.

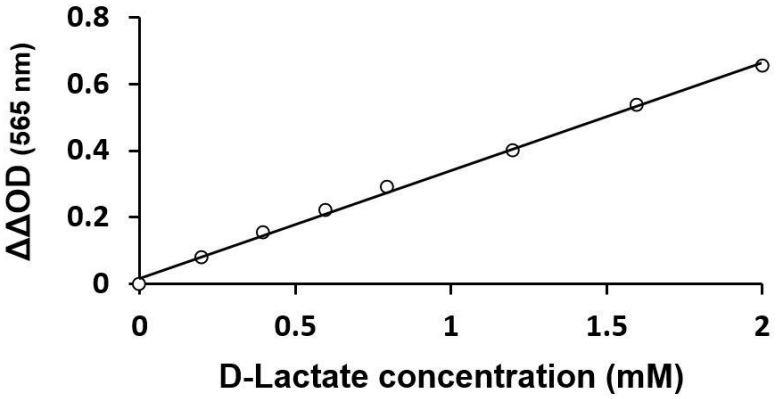
	Blank well	Standard well	Sample well
Each Standard		20 µL	
Each Sample	20 µL		20 µL
Working Reagent		80 µL	80 µL
Blank Working Reagent	80 µL		
Tap plate to mix briefly and thoroughly.			
Read the absorbance at **O.D. 565 nm** at **0** and **20 minutes incubation** at **room temperature. (OD_0 and OD_{20})**			

CALCULATION OF RESULTS

1. Subtract OD_0 from OD_{20} for the standard and sample wells. Use the ΔOD values to determine the sample D-lactate concentration from the standard curve. For samples requiring a No Enzyme A control (Blank), subtract the ΔOD_{BLANK} value from the ΔOD_{Sample} and use this ΔΔOD value to determine the sample D-lactate concentration from the standard curve.
2. If the sample OD value is higher than OD for 2 mM D-lactate standard, dilute sample in distilled water and repeat the assay. Multiply the results by the dilution factor.

EXAMPLE OF TYPICAL STANDARD CURVE

The following figures demonstrate typical results with the Lactate (D-Lactate) Assay Kit. One should use the data below for reference only. This data should not be used to interpret actual results.



QUALITY ASSURANCE

Sensitivity

0.05 mM