D-Lactate Assay Kit (Colorimetric)

LS-K236-100 (100 Tests) • **Store at -20°C**

Introduction

Lactate is generated by lactate dehydrogenase (LDH) under hypoxic or anaerobic conditions. Monitoring lactate levels is, therefore, a good indicator of the balance between tissue oxygen demand and utilization and is useful when studying cellular and animal physiology. D-lactate is produced in only minor quantities in animals and measuring for D-lactate in animal samples is a means to determine the presence of bacterial infection.

Simple, direct and automation-ready procedures for measuring lactate concentration are very desirable. LSBio's lactate assay 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 565 nm, is proportionate to the lactate concentration in the sample.

Key Features

- Sensitive and accurate. Detection limit of 0.05 mM and linearity up to 2 mM D-lactate in 96-well plate assay. For cell culture samples containing phenol red: detection limit of 0.1 mM and linearity up to 1 mM D-lactate in 96-well plate assay.
- Convenient. The procedure involves adding a single working reagent, and reading the optical density at time zero and at 20 min. Room temperature assay. No 37°C heater is needed.
- High-throughput. Can be readily automated as a high-throughput 96-well plate assay for thousands of samples per day.

Applications

- Direct Assays: D-lactate in serum, plasma, and cell media samples.

Components

Component	K236-100
	100 Tests
Assay Buffer	10 mL
Enzyme A	120 µL
Enzyme B	120 µL
NAD Solution	1 mL
MTT Solution	1.5 mL
Standard (20 mM D-lactate)	1.0 mL

Materials Not Supplied

Pipetting (multi-channel) devices. Clear-bottom 96-well plates (e.g. Corning Costar) and plate reader.

Storage

The kit is shipped on ice. Store all kit components at -20 °C.

D-Lactate Assay Kit (Colorimetric)

LS-K236-100 (100 Tests) • **Store at -20°C**

LSBio
LifeSpan BioSciences, Inc.

Assay Procedure

Important: 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 pipette is recommended.

1. Standard Curve. Prepare 1000 µL 2.0 mM D-lactate Premix by mixing 100 µL 20 mM Standard and 900 µL distilled water. For cell culture samples containing phenol red, prepare 1000 µL 1.0 mM lactate Premix by mixing 50 µL 20 mM Standard and 950 µL culture medium without serum. Dilute standard as follows. Transfer 20 µL standards into wells of a clear bottom 96-well plate.

No	Premix + H_2O or Medium	Vol (µL)	D-lactate (mM)
1	100µL + 0µL	100	2.0 or 1.0
2	80µL + 20µL	100	1.6 or 0.8
3	60µL + 40µL	100	1.2 or 0.6
4	40µL + 60µL	100	0.8 or 0.4
5	30µL + 70µL	100	0.6 or 0.3
6	20µL + 80µL	100	0.4 or 0.2
7	10µL + 90µL	100	0.2 or 0.1
8	0µL + 100µL	100	0

Samples. Add 20 µL sample per well in separate wells. For samples with potential endogenous enzyme activity (i.e. serum, plasma, tissue extracts), two reactions should be run: one with added Enzyme A and a No Enzyme A control. Serum and Plasma should be diluted at least 2× with dH_2O prior to assay.

2. Reagent Preparation. Spin the Enzyme tubes briefly before pipetting. For each Sample and Standard well, prepare Working Reagent by mixing 60 µL Assay Buffer, 1 µL Enzyme A, 1 µL Enzyme B, 10 µL NAD and 14 µL MTT. Fresh reconstitution is recommended. For the No Enzyme A control, the Working Reagent includes 60 µL Assay Buffer, 1 µL Enzyme B, 10 µL NAD and 14 µL MTT.

3. Reaction. Add 80 µL Working Reagent per reaction well quickly. Tap plate to mix briefly and thoroughly.

4. Read optical density (OD_0) for time "zero" at 565 nm (520-600nm) and OD_{20} after a 20-min incubation at room temperature.

5. Calculation. Subtract OD_0 from OD_{20} for the standard and sample wells. Use the D_{OD} values to determine the sample D-lactate concentration from the standard curve. For samples requiring a No Enzyme A control, subtract the ΔOD_{NoEnz} value from the ΔOD_{Sample} and use this $\Delta\Delta OD$ value to determine the sample D-lactate concentration from the standard curve.

Note: if the sample OD value is higher than OD for 2 mM D-lactate standard, dilute sample in water and repeat the assay. Multiply the results by the dilution factor.

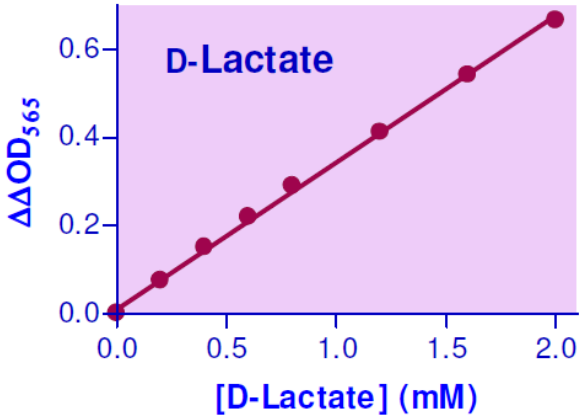
GENERAL CONSIDERATIONS

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%).

D-Lactate Assay Kit (Colorimetric)

LS-K236-100 (100 Tests) • **Store at -20°C**

Sample Data



Standard Curve in 96-well plate assay in water.

Version: V.08.09.2018