EnzyChrom™ L-Lactate Assay Kit (ECLC-100)

Colorimetric Determination of L-Lactate at 565 nm

DESCRIPTION

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.

Simple, direct and automation-ready procedures for measuring lactate concentration are very desirable. BioAssay Systems' EnzyChrom™ 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.

APPLICATIONS

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

KEY FEATURES

Sensitive and accurate. Detection limit of 0.05 mM and linearity up to 2 mM L-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 L-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.

KIT CONTENTS (100 tests in 96-well plates)

Assay Buffer: 10 mL	**NAD Solution: 1 mL**
Enzyme A: 120 µL	**MTT Solution: 1.5 mL**
Enzyme B: 120 µL	**Standard: 1.0 mL 20 mM L-Lactate**

Storage conditions. Store all reagents at -20°C. Shelf life: 6 months after receipt.

Precautions: 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.

PROCEDURES

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 pipettor is recommended.

1. *Standard Curve*. Prepare 1000 µL 2.0 mM L-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)	L-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 the 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 sample 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 ΔOD values to determine the sample L-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 ΔΔOD value to determine the sample L-lactate concentration from the standard curve.

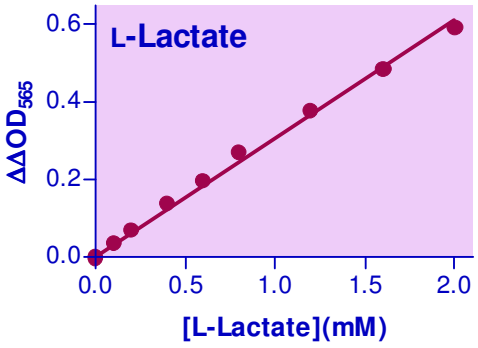
Note: if the sample OD value is higher than OD for 2 mM L-lactate standard, dilute sample in water and repeat the assay. Multiply the results by the dilution factor.

MATERIALS REQUIRED, BUT NOT PROVIDED

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

GENERAL CONSIDERATIONS

The following substances interfere and should be avoided in sample preparation: ascorbic acid, SDS (>0.2%), sodium azide, NP-40 (>1%) and Tween-20 (>1%).



Standard Curve in 96-well plate assay in water.

PUBLICATIONS

1. Senadheera D et al (2009). Inactivation of VicK affects acid production and acid survival of Streptococcus mutans. J Bacteriol. 191(20):6415-24.
2. Le Nihouannen D et al (2009). Ascorbic acid accelerates osteoclast formation and death. Bone 46(5):1336-43.
3. Milovanova TN et al (2008). Lactate stimulates vasculogenic stem cells via the thioredoxin system and engages an autocrine activation loop involving hypoxia-inducible factor 1. Mol Cell Biol. 28(20):6248-61.