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. The kit is shipped on ice. 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 μ L 2.0 mM L-lactate Premix by mixing 100 μ L 20 mM Standard and 900 μ L distilled water. For cell culture samples containing phenol red, prepare 1000 μ L 1.0 mM lactate Premix by mixing 50 μ L 20 mM Standard and 950 μ L culture medium without serum. Dilute standard as follows. Transfer 20 μ L standards into wells of a clear bottom 96-well plate.

No	Premix + H ₂ O or Medium	Vol (µL)	L-Lactate (mM)
1	100μL + ΟμL	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 μ L 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 dH₂O prior to the assay.

- 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 μ L Working Reagent per reaction well quickly. Tap plate to mix briefly and thoroughly.
- 4. Read optical density (OD₀) for time "zero" at 565 nm (520-600nm) and OD₂₀ after a 20-min incubation at room temperature.
- 5. Calculation. Subtract OD₀ from OD₂₀ for the standard and sample wells. Use the Δ OD values to determine the sample L-lactate concentration from the standard curve. For samples requiring a No Enzyme A control, subtract the Δ OD_{NoEnz} value from the Δ OD_{Sample} and use this Δ \DeltaOD 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. Aranda-Diaz, A., et al (2020). Bacterial interspecies interactions modulate pH-mediated antibiotic tolerance. ELife, 9.
- 2. Weis, Sebastian, et al (2017). Metabolic adaptation establishes disease tolerance to sepsis. Cell 169.7: 1263-1275.
- Chen, Z.-T., et al (2020). Long non-coding RNA Linc00092 inhibits cardiac fibroblast activation by altering glycolysis in an ERKdependent manner. Cellular Signalling, 74, 109708.

