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Product Information

L-Lactate Assay Kit

Catalog Number **MAK329**
Storage Temperature –20 °C

TECHNICAL BULLETIN

Product 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. The 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.

Detection limit of 0.05 mM and linearity up to 2 mM L-Lactate in the 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 using the 96 well plate assay.

This kit is suitable for lactate determination in serum, plasma, and cell media samples.

Components
The kit is sufficient for 100 colorimetric assays in 96 well plates.

Assay Buffer Catalog Number MAK329A	10 mL
Enzyme A Catalog Number MAK329B	120 µL
Enzyme B Catalog Number MAK329C	120 µL
NAD Solution Catalog Number MAK329D	1 mL
MTT Solution Catalog Number MAK329E	1.5 mL
Standard (20 mM L-Lactate) Catalog Number MAK329F	1 mL

Reagents and Equipment Required but Not Provided.
- Pipetting devices and accessories (e.g., multichannel pipettor)
- Centrifuge tubes
- 96 well flat bottom plate. It is recommended to use clear plates for colorimetric assays
- Spectrophotometric multiwell plate reader

Precautions and Disclaimer
This product is for R&D use only, not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability
The kit is shipped on dry ice. Store all components at –20 °C upon receiving.

Procedure
Sample Preparation
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 control reaction with No Enzyme A.

Serum and Plasma should be diluted at least 2× with ultrapure water prior to the assay.

The following substances interfere with the assay and should be avoided in sample preparation: ascorbic acid, sodium dodecyl sulfate (>0.2%), sodium azide, NP-40 (>1%) and TWEEN® 20 (>1%).

Standard Curve

Prepare 1,000 µL of 2.0 mM L-Lactate Premix by mixing 100 µL of 20 mM Standard and 900 µL of ultrapure water. For cell culture samples containing phenol red, prepare 1,000 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 according to Table 1 (samples excluding cell culture containing phenol red) or Table 2 (cell culture samples containing phenol red).

Table 1.
Preparation of L-Lactate Standards for all samples excluding cell culture samples containing phenol red

Tube	Premix	Ultrapure Water	L-Lactate (mM)
1	100 µL	0 µL	2.0
2	80 µL	20 µL	1.6
3	60 µL	40 µL	1.2
4	40 µL	60 µL	0.8
5	30 µL	70 µL	0.6
6	20 µL	80 µL	0.4
7	10 µL	90 µL	0.2
8	0 µL	100 µL	0

Table 2.
Preparation of L-Lactate Standards for cell culture samples containing phenol red

Tube	Premix	Culture Medium	L-Lactate (mM)
1	100 µL	0 µL	1.0
2	80 µL	20 µL	0.8
3	60 µL	40 µL	0.6
4	40 µL	60 µL	0.4
5	30 µL	70 µL	0.3
6	20 µL	80 µL	0.2
7	10 µL	90 µL	0.1
8	0 µL	100 µL	0

Reaction Mix

Note: Briefly centrifuge enzyme tubes before opening. For each Sample and Standard well, prepare Reaction Mix by mixing:

60 µL of Assay Buffer
1 µL of Enzyme A
1 µL of Enzyme B
10 µL of NAD
14 µL of MTT

Fresh preparation just prior to use is recommended.

For the samples which are control reactions with No Enzyme A (see Sample Preparation, the Reaction Mix includes:

60 µL of Assay Buffer
1 µL of Enzyme B
10 µL of NAD
14 µL of MTT

Assay Reaction

1. Transfer 20 µL of standards into separate wells of a clear, flat bottom 96 well plate.
2. Transfer 20 µL of each sample into separate wells.
3. Add 80 µL of Reaction Mix per reaction well quickly. Tap plate to mix briefly and thoroughly.
   Note: This assay is based on an enzyme-catalyzed kinetic reaction. Addition of Reaction Mix should be quick and mixing should be brief but thorough. Use of a multichannel pipettor is recommended.
4. Immediately measure the initial absorbance at 565 nm (A_{565}).
5. Incubate plate for 20 minutes at room temperature.
6. Measure the final absorbance at 565 nm (A_{565}).

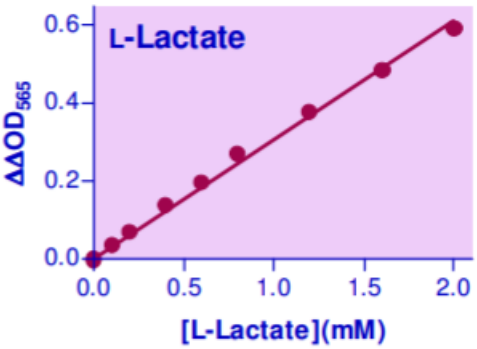
Results

Subtract initial A_{565} from the final A_{565} for the standard and sample wells. Use the ΔA_{565} values to determine the sample L-lactate concentration from the standard curve.

For samples requiring a No Enzyme A control reaction, subtract the ΔA_{565} No Enzyme value from the ΔA_{565} Sample and use this value to determine the sample L-lactate concentration from the standard curve.

Note: If the sample A_{565} value is higher than A_{565} for 2 mM L-lactate standard, dilute sample in ultrapure water and repeat the assay. Multiply the results by the dilution factor.

Figure 1.
Typical Standard Curve



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