SIGMA-ALDRICH®

sigma-aldrich.com

3050 Spruce Street, St. Louis, MO 63103 USA
Tel: (800) 521-8956 (314) 771-5765 Fax: (800) 325-5052 (314) 771-5757
email: techservice@sial.com sigma-aldrich.com

Product Information

D-Lactate Assay Kit

Catalog Number **MAK336**
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. 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. The D-Lactate Assay Kit is based on lactate dehydrogenase catalyzed oxidation of lactate, in which the formed NADH reduces MTT to the formazan chromogen. The intensity of the product color, measured at 565 nm, is proportional to the lactate concentration in the sample.

For the 96 well plate assay, the detection limit is 0.05 mM with linearity up to 2 mM D-lactate. For cell culture samples containing phenol red, the detection limit is 0.1 mM with linearity up to 1 mM D-lactate in the 96 well plate assay.

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

Components

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

Component	Amount
Assay Buffer Catalog Number MAK336A	10 mL
Enzyme A Catalog Number MAK336B	120 μL
Enzyme B Catalog Number MAK336C	120 μL
NAD Solution Catalog Number MAK336D	1 mL
MTT Solution Catalog Number MAK336E	1.5 mL
Standard (20 mM D-Lactate) Catalog Number MAK336F	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

<u>Sample Preparation</u>

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, EDTA (>0.5 mM), sodium dodecyl sulfate (>0.2%), sodium azide, NP-40 (>1%), and TWEEN® 20 (>1%).

Standard Curve

Prepare 1,000 µL of 2.0 mM D-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 D-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 D-Lactate Standards for all samples excluding cell culture samples containing phenol red

Tube	Premix	Ultrapure Water	D-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 D-Lactate Standards for cell culture samples containing phenol red

Tube	Premix	Culture Medium	D-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 Assay Buffer
1 µL Enzyme A
1 µL Enzyme B
10 µL NAD Solution
14 µL MTT Solution

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 Assay Buffer
1 µL Enzyme B
10 µL NAD Solution
14 µL MTT Solution

Assay Reaction

1. Transfer 20 µL 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}).

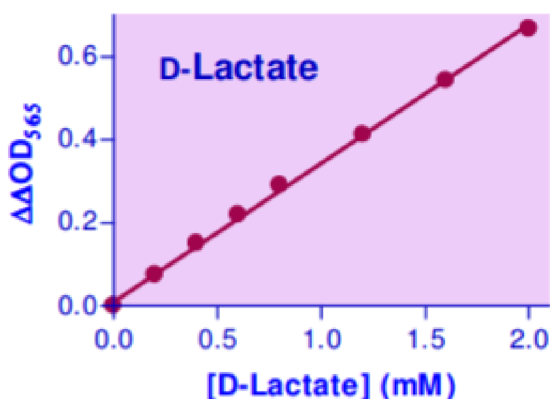
Results

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

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

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

Figure 1.
Typical Standard Curve



References

1. Babson, A.L., and Babson, S.R., Kinetic Colorimetric Measurement of Serum Lactate Dehydrogenase Activity. Clin. Chem., **19(7)**, 766-9 (1973).
2. Karlsen, R.L. et al., A rapid method for the determination of urea stable lactate dehydrogenase on the 'Cobas Bio' centrifugal analyser. Scand. J. Clin. Lab. Invest., **41(5)**, 513-6 (1981).
3. Coley, H.M. et al., Chemosensitivity testing of fresh and continuous tumor cell cultures using lactate dehydrogenase. Anticancer Res., 17(1A), 231-6 (1997).

TWEEN is a registered trademark of Croda International PLC

HM,MAM 11/18-1

©2018 Sigma-Aldrich Co. LLC. All rights reserved. SIGMA-ALDRICH is a trademark of Sigma-Aldrich Co. LLC, registered in the US and other countries. Sigma brand products are sold through Sigma-Aldrich, Inc. Purchaser must determine the suitability of the product(s) for their particular use. Additional terms and conditions may apply. Please see product information on the Sigma-Aldrich website at www.sigmaaldrich.com and/or on the reverse side of the invoice or packing slip.