
Product Manual

Lactate Assay Kit (Colorimetric)

Catalog Number

MET-5012

100 assays

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures



CELL BIOLABS, INC.
Creating Solutions for Life Science Research

Introduction

Lactic Acid is an alpha hydroxyl acid that can ionize a carboxyl proton to yield the lactate ion, the latter of which exists as two optical isomers L-Lactate and D-Lactate. The enzyme lactate dehydrogenase catalyzes the conversion of pyruvate to lactate in animals during the process of fermentation. Depending on the levels of exercise, blood levels of lactate can vary between 1 and 20 mM.

In medicine, lactate is a component of intravenous fluids such as Hartmann's solution. These fluids are often used when blood loss occurs due to surgery or injury. In the brain, lactate, like glucose, is thought to be one of the main sources of energy. High levels of lactate have been found in the extracellular fluid surrounding neurons due to the high metabolic activity of glial cells. In the food industry, lactic acid is found in cheeses, milk, and various breads. In winemaking, lactic acid bacteria are used to reduce malic acid levels and therefore decrease the sharpness in flavor. Finally, in the detergent industry lactic acid has been used as an anti-bacterial agent as well as a soap-scum removal agent and descaler.

Cell Biolabs' Lactate Assay Kit (Colorimetric) is a simple colorimetric assay that measures the total amount of L-lactate present in biological samples in a 96-well microtiter plate format. Lactate is oxidized by lactate oxidase into pyruvate and hydrogen peroxide. The hydrogen peroxide is then detected with a highly specific colorimetric probe. Horseradish peroxidase catalyzes the reaction between the probe and hydrogen peroxide, which bind in a 1:1 ratio. Samples and standards are incubated for 30-45 minutes and then read with a standard 96-well colorimetric plate reader (Figure 1). Samples are compared to a known concentration of lactate standard within the 96-well microtiter plate format. Each kit provides sufficient reagents to perform up to 100 assays, including blanks, lactate standards and unknown samples. Sample lactate concentrations are determined by comparison with a known lactate standard. The kit has a detection sensitivity limit of 1.5 μ M L-Lactate.

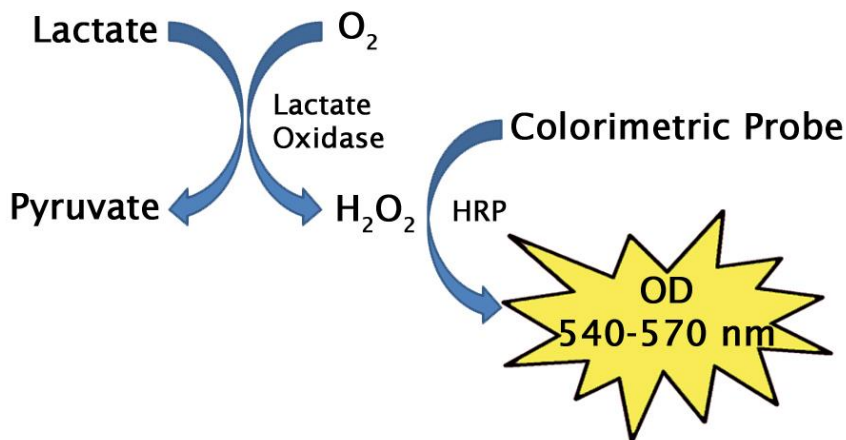


Figure 1. Lactate assay principle.

Related Products

1. MET-5001: Lactose Assay Kit (Fluorometric)
2. MET-5013: Lactate Assay Kit (Fluorometric)
3. MET-5014: NAD⁺/NADH Assay Kit (Colorimetric)
4. MET-5151: S-Adenosylhomocysteine (SAH) ELISA Kit
5. MET-5152: S-Adenosylmethionine (SAM) ELISA Kit
6. STA-398: Free Glycerol Assay Kit (Colorimetric)
7. STA-674: Glutamate Assay Kit
8. STA-675: Hydroxyproline Assay Kit
9. STA-680: Glucose Assay Kit (Colorimetric)
10. STA-682: Total Carbohydrate Assay Kit

Kit Components

1. Lactate Standard (Part No. 50121C): One 100 μ L tube at 100 mM
2. 10X Assay Buffer (Part No. 234403): One 25 mL bottle
3. Colorimetric Probe (Part No. 50122C): One 50 μ L amber tube
4. HRP (Part No. 234402): One 100 μ L tube at 100 U/mL in glycerol
5. Lactate Oxidase (Part No. 50123C): One 30 μ L tube at 200 U/mL

Note: One unit is defined as the amount of enzyme that will oxidize 1.0 micromole of L-Lactate to pyruvate and hydrogen peroxide per minute at pH 7 at 37°C.

Materials Not Supplied

1. Distilled or deionized water
2. 1X PBS
3. 10 μ L to 1000 μ L adjustable single channel micropipettes with disposable tips
4. 50 μ L to 300 μ L adjustable multichannel micropipette with disposable tips
5. Standard 96-well clear microtiter plate and/or clear cell culture microplate
6. Multichannel micropipette reservoir
7. Spectrophotometric microplate reader capable of reading in the 540-570 nm range.

Storage

Upon receipt, store the 10X Assay Buffer at 4°C. Store all remaining components at -20°C. The Colorimetric Probe is light sensitive and must be stored accordingly. Avoid multiple freeze/thaw cycles.

Preparation of Reagents

- 1X Assay Buffer: Dilute the stock 10X Assay Buffer 1:10 with deionized water for a 1X solution. Stir or vortex to homogeneity.
- Reaction Mix: Prepare a Reaction Mix by diluting the Colorimetric Probe 1:100, HRP 1:500, and Lactate Oxidase 1:200 in 1X Assay Buffer. For example, add 10 μ L Colorimetric Probe stock solution, 2 μ L HRP stock solution, and 5 μ L of Lactate Oxidase to 983 μ L of 1X Assay Buffer for a total of 1 mL. This Reaction Mix volume is enough for 20 assays. The Reaction Mix is stable for 1 day at 4°C.

Note: Prepare only enough for immediate use by scaling the above example proportionally.

Preparation of Samples

- Cell culture supernatants: Cell culture media containing lactate should be avoided. To remove insoluble particles, centrifuge at 10,000 rpm for 5 min. The cell conditioned media may be assayed directly or diluted as necessary. Prepare the Lactate standard curve in non-conditioned media without lactate.

Note: Maintain pH between 7 and 8 for optimal working conditions as the Colorimetric Probe is unstable at high pH (>8.5).

- Tissue lysates: Sonicate or homogenize tissue sample in cold PBS or 1X Assay Buffer and centrifuge at 10000 x g for 10 minutes at 4°C. Perform dilutions in 1X Assay Buffer.
- Cell lysates: Resuspend cells at 1-2 x 10⁶ cells/mL in PBS or 1X Assay Buffer. Homogenize or sonicate the cells on ice. Centrifuge to remove debris. Cell lysates may be assayed undiluted or diluted as necessary in 1X Assay Buffer.
- Serum, plasma, saliva, or urine: To remove insoluble particles, centrifuge at 10,000 rpm for 5 min. The supernatant may be assayed directly or diluted as necessary in 1X Assay Buffer.

Notes:

- *All samples should be assayed immediately or stored at -80°C for up to 1-2 months. Run proper controls as necessary. Optimal experimental conditions for samples must be determined by the investigator. Always run a standard curve with samples.*
- *Samples with NADH concentrations above 10 μ M and glutathione concentrations above 50 μ M will oxidize the Colorimetric Probe and could result in erroneous readings. To minimize this interference, it is recommended that superoxide dismutase (SOD) be added to the reaction at a final concentration of 40 U/mL (Votyakova and Reynolds, Ref. 2).*
- *Avoid samples containing DTT or β -mercaptoethanol since the Colorimetric Probe is not stable in the presence of thiols (above 10 μ M).*

Preparation of Standard Curve

Prepare fresh Lactate standards before use by diluting in 1X Assay Buffer. First, dilute the stock 100 mM Lactate Standard solution 1:10 in 1X Assay Buffer to yield a 10 mM Lactate Solution (e.g. add 5 μL of the stock 100 mM Lactate Standard to 45 μL of 1X Assay Buffer). Use the 10 mM Lactate Solution to prepare a series of the remaining Lactate standards according to Table 2 below.

Standard Tubes	10 mM Lactate Solution (μL)	1X Assay Buffer (μL)	Lactate (μM)	Lactate (mg/dL)
1	10	990	100	1.12
2	250 of Tube #1	250	50	0.56
3	250 of Tube #2	250	25	0.28
4	250 of Tube #3	250	12.5	0.14
5	250 of Tube #4	250	6.25	0.07
6	250 of Tube #5	250	3.13	0.035
7	250 of Tube #6	250	1.56	0.0175
8	0	250	0	0

Table 2. Preparation of Lactate Standards

Assay Protocol

1. Prepare and mix all reagents thoroughly before use. Each sample, including unknowns and standards, should be assayed in duplicate or triplicate.
2. Add 50 μL of each lactate standard or unknown sample into wells of a 96-well microtiter plate.
3. Add 50 μL of Reaction Mix to each well. Mix the well contents thoroughly and incubate for 30-45 minutes at 37°C protected from light.

Note: This assay is continuous (not terminated) and therefore may be measured at multiple time points to follow the reaction kinetics.

4. Read the plate with a spectrophotometric microplate reader in the 540-570 nm range.
5. Calculate the concentration of lactate within samples by comparing the sample OD to the standard curve.

Example of Results

The following figures demonstrate typical Lactate Assay Kit (Colorimetric) results. One should use the data below for reference only. This data should not be used to interpret or calculate actual sample results.

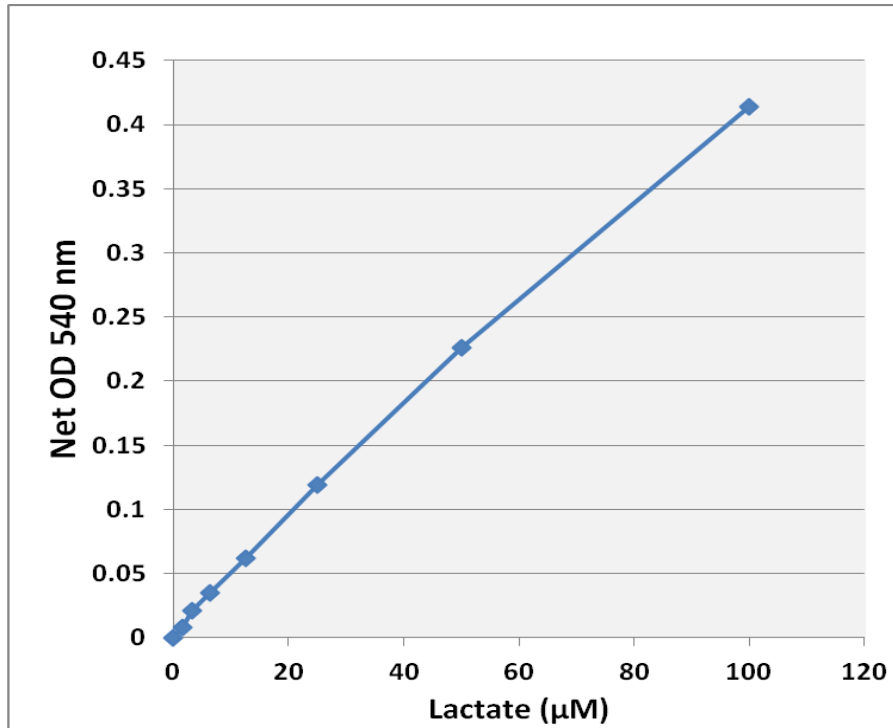


Figure 2: Lactate standard curve.

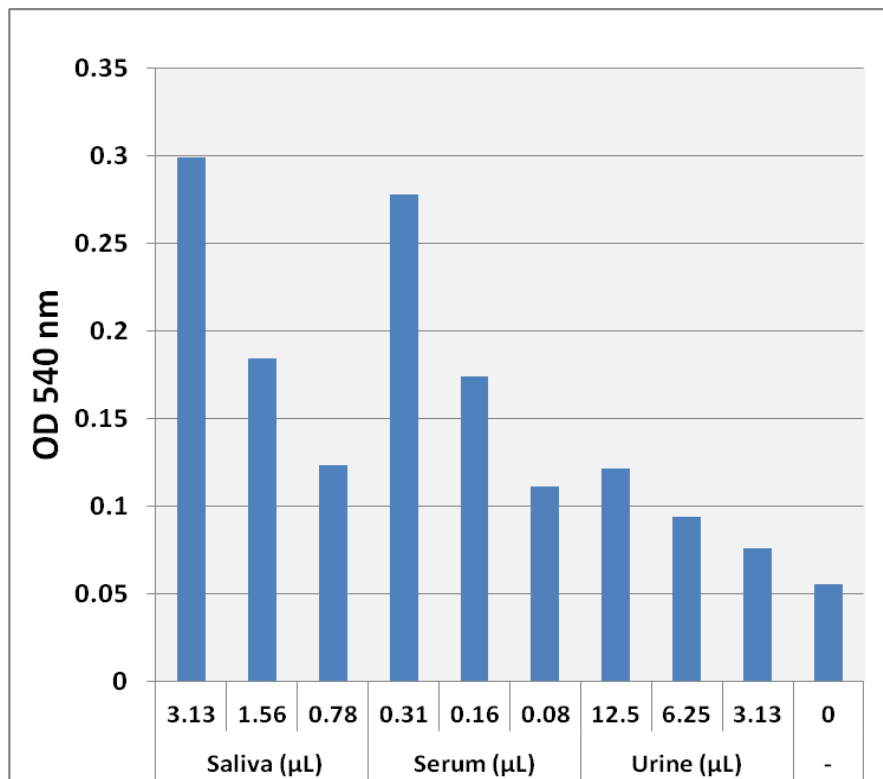


Figure 3: Lactate detection in human saliva, serum, or urine using the Lactate Assay Kit (Colorimetric).

References

1. Connor H and Woods HF *Ciba Found Symp.*(1982) **87**:214-234.
2. Votyakova TV, and Reynolds IJ (2001) *Neurochem.* **79**:266.
3. Robergs, RA; Ghiasvand, F; Parker, D (2004). *Am J Physiol Regul Integr Comp Physiol* **287**: R502–R516.
4. Wyss MT, Jolivet R, Buck A, Magistretti PJ, Weber B (2011). *J. Neurosci.* **31**: 7477–85.
5. Holmgren CD, Mukhtarov M, Malkov AE, Popova IY, Bregestovski P, Zilberter Y (2010). . *J. Neurochem.* **112**: 900–12

Recent Product Citations

1. Meléndez, D.M. et al. (2022). Effect of rest, post-rest transport duration, and conditioning on performance, behavioural, and physiological welfare indicators of beef calves. *PLoS One.* **17**(12):e0278768. doi: 10.1371/journal.pone.0278768.
2. Amrutkar, M. et al. (2022). Neoadjuvant chemotherapy is associated with an altered metabolic profile and increased cancer stemness in patients with pancreatic ductal adenocarcinoma. *Mol Oncol.* doi: 10.1002/1878-0261.13344.
3. Stefan, J. et al. (2021). Differential and Overlapping Effects of Melatonin and Its Metabolites on Keratinocyte Function: Bioinformatics and Metabolic Analyses. *Antioxidants.* **10**(4):618. doi: 10.3390/antiox10040618.
4. Meléndez, D.M. et al. (2021). Correlation between L-Lactate Concentrations in Beef Cattle, Obtained Using a Hand-Held Lactate Analyzer and a Lactate Assay Colorimetric Kit. *Animals (Basel).* **11**(4):926. doi: 10.3390/ani11040926.
5. Meléndez, D.M. et al. (2021). Effects of conditioning, source, and rest on indicators of stress in beef cattle transported by road. *PLoS One.* **16**(1):e0244854. doi: 10.1371/journal.pone.0244854.
6. Rattu, G. et al. (2020). Lactate detection sensors for food, clinical and biological applications: a review. *Environ Chem Lett.* doi: 10.1007/s10311-020-01106-6.
7. Lomeli, M.J.M. et al. (2020). Use of artificial illumination to reduce Pacific halibut bycatch in a U.S. West Coast groundfish Bottom trawl. *Fish Res.* doi: 10.1016/j.fishres.2020.105737.
8. Aldonza, M. B. D. et al. (2020). Prior acquired resistance to paclitaxel relays diverse EGFR-targeted therapy persistence mechanisms. *Sci. Adv.* **6**(6): eaav7416. doi: 10.1126/sciadv.aav7416.
9. Gospe, S.M. et al. (2019). Photoreceptors in a Mouse Model of Leigh Syndrome are Capable of Normal Light-Evoked Signaling. *J Biol Chem.* pii: jbc.RA119.007945. doi: 10.1074/jbc.RA119.007945.
10. Guo, R. et al. (2018). Cardiomyocyte-specific disruption of Cathepsin K protects against doxorubicin-induced cardiotoxicity. *Cell Death Dis.* **9**(6):692. doi: 10.1038/s41419-018-0727-2.
11. Longchamp, A. et al. (2018). Amino Acid Restriction Triggers Angiogenesis via GCN2/ATF4 Regulation of VEGF and H2S Production. *Cell.* **173**(1):117-129.e14. doi: 10.1016/j.cell.2018.03.001.

Warranty

These products are warranted to perform as described in their labeling and in Cell Biolabs literature when used in accordance with their instructions. THERE ARE NO WARRANTIES THAT EXTEND BEYOND THIS EXPRESSED WARRANTY AND CELL BIOLABS DISCLAIMS ANY IMPLIED WARRANTY OF MERCHANTABILITY OR WARRANTY OF FITNESS FOR PARTICULAR PURPOSE. CELL BIOLABS's sole obligation and purchaser's exclusive remedy for breach of this warranty shall be, at the option of CELL BIOLABS, to repair or replace the products. In

no event shall CELL BIOLABS be liable for any proximate, incidental or consequential damages in connection with the products.

Contact Information

Cell Biolabs, Inc.
7758 Arjons Drive
San Diego, CA 92126
Worldwide: +1 858 271-6500
USA Toll-Free: 1-888-CBL-0505
E-mail: tech@cellbiolabs.com
www.cellbiolabs.com

©2016-2023: Cell Biolabs, Inc. - All rights reserved. No part of these works may be reproduced in any form without permissions in writing.