Product Manual

Lactate Assay Kit (Fluorometric)

Catalog Number

MET-5013 **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 (Fluorometric) is a simple fluorometric assay that measures the total amount of L-lactate present in biological samples in a 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 µM L-Lactate.

Assay Principle

Cell Biolabs' Lactate Assay Kit measures L-lactate within biological samples. Lactate is oxidized by lactate oxidase into pyruvate and hydrogen peroxide. The hydrogen peroxide is then detected with a highly specific Fluorometric 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 Fluorometric plate reader (Figure 1). Samples are compared to a known concentration of lactate standard within the 96-well microtiter plate format.

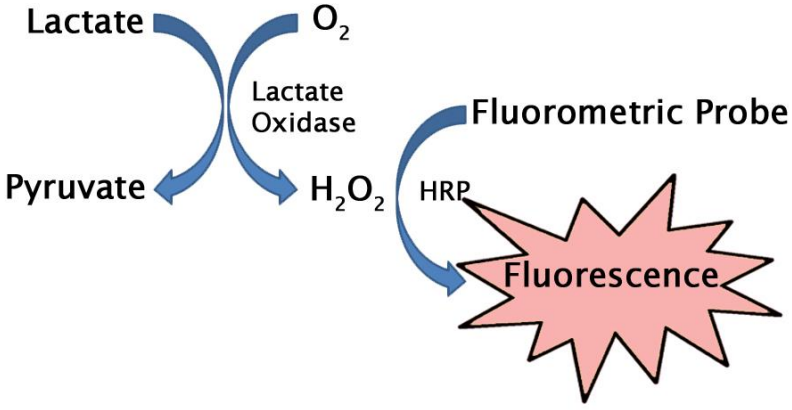

Figure 1. Lactate assay principle.

Related Products

1. MET-5029: Pyruvate Assay Kit (Fluorometric)
2. STA-681: Glucose Assay Kit (Fluorometric)
3. MET-5023: Glycogen Assay Kit (Fluorometric)

4. MET-5030: NAD+/NADH Assay Kit (Fluorometric)
5. MET-5163: ATP Assay Kit (Fluorometric)

Kit Components

1. Lactate Standard (Part No. 50121C): One 100 µL tube at 100 mM
2. 10X Assay Buffer (Part No. 234403): One 25 mL bottle
3. Fluorometric Probe (Part No. 50131C): One 50 µL amber tube
4. HRP (Part No. 234402): One 100 µL tube at 100 U/mL in glycerol
5. Lactate Oxidase (Part No. 50123C): One 30 µL 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. Standard 96-well clear microtiter plate and/or clear cell culture microplate

Storage

Upon receipt, store the 10X Assay Buffer at 4ºC. Store all remaining components at -20ºC. The Fluorescence 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 Fluorometric Probe 1:100, HRP 1:500, and Lactate Oxidase 1:200 in 1X Assay Buffer. For example, add 10 µL Fluorometric Probe stock solution, 2 µL HRP stock solution, and 5 µL of Lactate Oxidase to 983 µL 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 4ºC.
  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 Fluorometric 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 10^6 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 µM and glutathione concentrations above 50 µM will oxidize the Fluorometric 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 Fluorometric Probe is not stable in the presence of thiols (above 10 µM).*

Preparation of Standard Curve

1. 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). Vortex thoroughly.
2. Use the 10 mM Lactate Solution to prepare a series of the remaining Lactate standards according to Table 2.

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 37ºC 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 fluorescence microplate reader equipped for excitation in the 530-570 nm range and for emission in the 590-600 nm range.
5. Calculate the concentration of lactate within samples by comparing the sample RFU to the lactate standard curve.

Example of Results

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

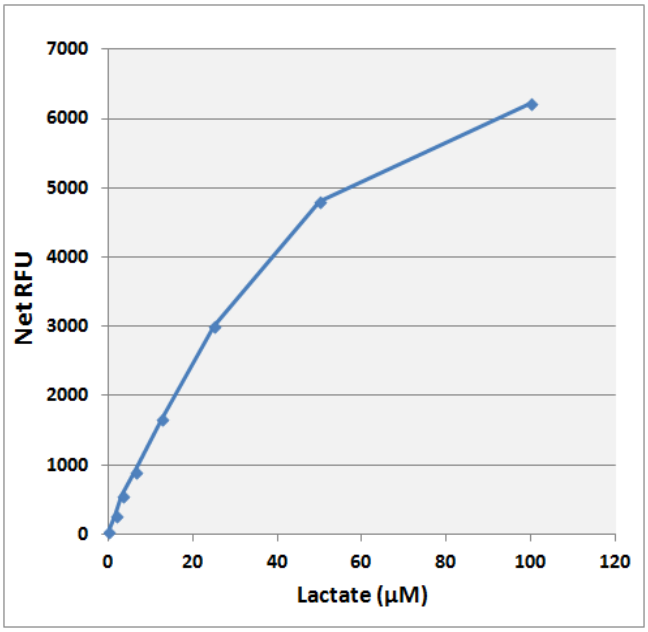

Figure 2. Lactate Standard Curve

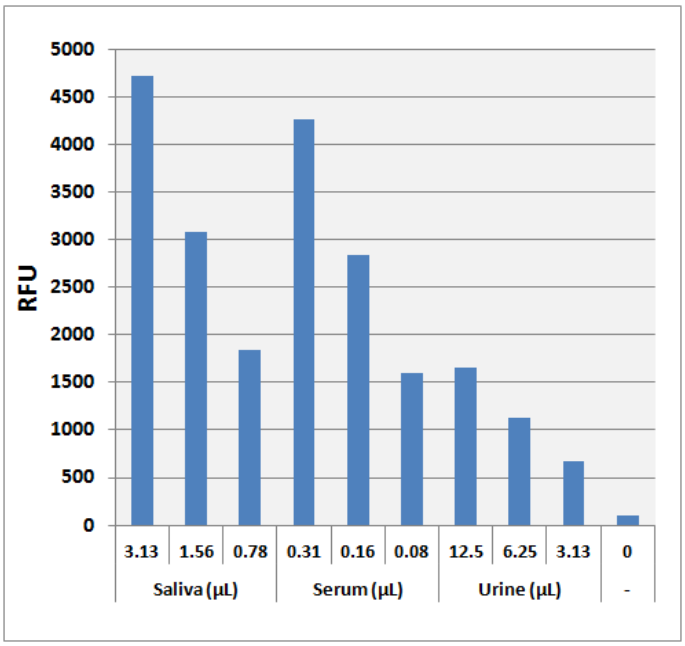

Figure 3: Lactate detection in human saliva, serum, or urine using the Lactate Assay Kit (Fluorometric).

References

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Recent Product Citations

1. Kaneko, Y. et al. (2020). Rhynchophylline promotes stem cell autonomous metabolic homeostasis. *Cytotherapy*. pii: S1465-3249(19)30925-9. doi: 10.1016/j.jcyt.2019.12.008.
2. Ogando, J. et al. (2019). PD-1 signaling affects cristae morphology and leads to mitochondrial dysfunction in human CD8+ T lymphocytes. *J Immunother Cancer*. **7**(1):151. doi: 10.1186/s40425-019-0628-7.

Warranty

Contact Information

Cell Biolabs, Inc.
5628 Copley Drive
San Diego, CA 92111
Worldwide: +1 858 271-6500
USA Toll-Free: 1-888-CBL-0505
E-mail: tech@cellbiolabs.com
www.cellbiolabs.com