Amplite™ Fluorimetric D-Lactate Assay Kit

Catalog number: 13810
Unit size: 200 Tests

Component	Storage	Amount
Component A: Enzyme Mix	Freeze (<-15 °C), Minimize light exposure	2 bottles (lyophilized powder)
Component B: Assay Buffer	Freeze (<-15 °C)	1 bottle (10 mL)
Component C: NAD	Freeze (<-15 °C), Minimize light exposure	1 vial
Component D: D-Lactate Standard	Freeze (<-15 °C), Minimize light exposure	2.25 mg/vial

OVERVIEW

Lactic acid is chiral and has two optical isomers: L-lactic acid and D-lactic acid. Lactate is constantly produced from pyruvate via the enzyme lactate dehydrogenase (LDH) in the process of metabolism and exercise. Monitoring lactate levels is a good way to evaluate the balance between tissue oxygen demand and utilization and is useful when studying cellular and animal physiology. D-lactate is not metabolized by mammals and its elimination from the body depends mainly on renal excretion. D- and L-lactic acid are found in many fermented milk products such as yogurt and cheese, and also in pickled vegetables, and cured meats and fish. The D- and L-lactic acid (generated by bacteria) is a quality indicator of foods, such as egg, milk, fruit juice and wine. Abnormal high concentration of D-lactate in the blood is usually a reflection of bacterial overgrowth in the gastrointestinal tract. AAT Bioquest's Amplite™ Lactate Assay Kits (Cat# 13814 and 13815 for L-lactate assay, and Cat# 13810 and 13811 for D-lactate assay) provide both fluorescence and absorbance-based method for detecting either L-lactate or D-lactate in biological samples such as serum, plasma, urine, as well as in cell culture samples. In the enzyme coupled assay, lactate is proportionally related to NADH, which is specifically monitored by a fluorogenic NADH sensor. The signal can be read by a fluorescence microplate reader. With this Fluorimetric Amplite™ D-Lactate Assay Kit, we were able to detect as little as 1.4 µM D-lactate in a 100 µL reaction volume.

AT A GLANCE

Protocol summary

1. Prepare D-Lactate working solution (50 µL)
2. Add D-Lactate standards or test samples (50 µL)
3. Incubate at room temperature for 30 min - 2 hours
4. Monitor fluorescence increase at Ex/Em = 540/590 nm (Cutoff = 570 nm)

Important Thaw one vial of each kit component at room temperature before starting the experiment.

KEY PARAMETERS

Instrument:	Fluorescence microplate reader
Excitation:	540 nm
Emission:	590 nm
Cutoff:	570 nm
Recommended plate:	Solid black

PREPARATION OF STOCK SOLUTIONS

Unless otherwise noted, all unused stock solutions should be divided into single-use aliquots and stored at -20 °C after preparation. Avoid repeated freeze-thaw cycles.

1. NAD stock solution (100X):
Add 100 µL of H_2O into the vial of NAD (Component C) to make 100X NAD stock solution.

2. D-Lactate standard solution (100 mM):
Add 200 µL of H_2O or 1x PBS buffer into the vial of D-Lactate Standard (Component D) to make 100 mM D-Lacate standard solution.

PREPARATION OF STANDARD SOLUTION

D-Lactate standard

For convenience, use the Serial Dilution Planner:
https://www.aatbio.com/tools/serial-dilution/13810

Add 10 µL of 100 mM D-Lactate standard solution into 990 µL 1x PBS buffer to generate 1 mM D-Lactate standard solution (SD7). Take 1 mM D-Lactate standard solution (SD7) and perform 1:3 serial dilutions in 1x PBS buffer to get serially diluted D-Lactate standards (SD6 - SD1).

Note Diluted D-Lactate standard solution is unstable, and should be used within 4 hours.

PREPARATION OF WORKING SOLUTION

1. Add 5 mL of Assay Buffer (Component B) into one bottle of Enzyme Mix (Component A), and mix well.

2. Add 50 µL of 100X NAD stock solution into the bottle of Component A+B, and mix well to make D-Lactate working solution.

Note This D-Lactate working solution is enough for one 96-well plate. Protect from light.

SAMPLE EXPERIMENTAL PROTOCOL

Table 1. Layout of D-Lactate standards and test samples in a solid black 96-well microplate. SD= D-Lactate Standards (SD1 - SD7, 1 to 1000 µM), BL=Blank Control, TS=Test Samples.

BL	BL	TS	TS
SD1	SD1	...	...
SD2	SD2	...	...
SD3	SD3		
SD4	SD4		
SD5	SD5		
SD6	SD6		
SD7	SD7		

Table 2. Reagent composition for each well.

Well	Volume	Reagent
SD1 - SD7	50 µL	Serial Dilutions (1 to 1000 µM)
BL	50 µL	Dilution Buffer
TS	50 µL	test sample

1. Prepare D-Lactate standards (SD), blank controls (BL), and test samples (TS)

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according to the layout provided in Tables 1 and 2. For a 384-well plate, use 25 µL of reagent per well instead of 50 µL.

2. Add 50 µL of D-Lactate working solution to each well of D-Lactate standard, blank control, and test samples to make the total D-Lactate assay volume of 100 µL/well. For a 384-well plate, add 25 µL of D-Lactate working solution into each well instead, for a total volume of 50 µL/well.

3. Incubate the reaction at room temperature for 30 minutes to 2 hours, protected from light.

4. Monitor the fluorescence increase with a fluorescence plate reader at Excitation = 530 - 570 nm, Emission = 590 - 600 nm (optimal Ex/Em = 540/590 nm, Cutoff = 570 nm).

EXAMPLE DATA ANALYSIS AND FIGURES

The reading (RFU) obtained from the blank standard well is used as a negative control. Subtract this value from the other standards' readings to obtain the base-line corrected values. Then, plot the standards' readings to obtain a standard curve and equation. This equation can be used to calculate D-Lactate samples. We recommend using the Online Linear Regression Calculator which can be found at:

https://www.aatbio.com/tools/linear-logarithmic-semi-log-regression-online-calculator

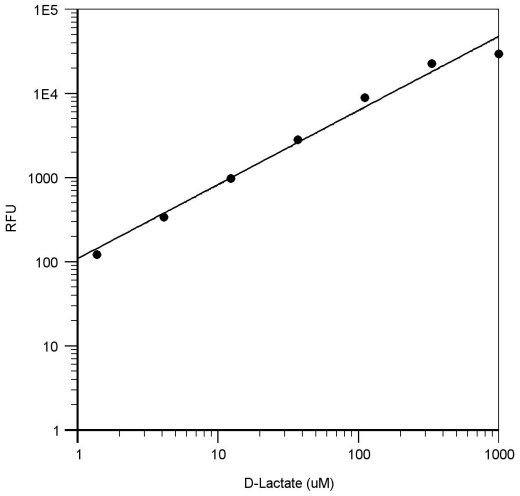

Figure 1. D-lactate dose response was measured with Amplite™ Fluorimetric D-Lactate Assay Kit in a 96-well solid black plate using a Gemini (Molecular Devices) microplate reader.

DISCLAIMER

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