Amplite™ Fluorimetric D-Lactate Dehydrogenase (LDH) Assay Kit

Catalog number: 13808
Unit size: 200 Tests

Component	Storage	Amount
Component A: Enzyme Probe	Freeze (<-15 °C), Minimize light exposure	1 bottle (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: LDH	Freeze (<-15 °C), Minimize light exposure	10 U/vial

OVERVIEW

Lactate dehydrogenase (LDH) is an oxidoreductase enzyme that catalyzes the interconversion of pyruvate and lactate. LDH is present in cytosol of a wide variety of organisms, including animals and plants. Cells release LDH into the bloodstream after tissue damage or red blood cell hemolysis. Since LDH is a fairly stable enzyme, it has been widely used to evaluate the presence of damage and toxicity of tissue and cells. Quantification of LDH has a broad range of applications. LDH is also elevated in certain pathological conditions such as cancer. This Amplite™ Lactate Dehydrogenase Assay Kit provides a fluorescence-based method for detecting D-lactate dehydrogenase (D-LDH) in biological samples such as serum, plasma, urine, as well as in cell culture samples. In the enzyme coupled assay, LDH is proportionally related to the concentration of NADH that is specifically monitored by a fluorogenic NADH sensor. This assay is specific for D-LDH. The fluorescence signal can be read by a fluorescence microplate reader. With this fluorimetric Amplite™ D-lactate Dehydrogenase Assay Kit, we were able to detect as little as 1 mU/mL D-lactate dehydrogenase in a 100 µL reaction volume.

AT A GLANCE

Protocol summary

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

Important Thaw one 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-LDH standard solution (100 U/mL):
Add 100 µL of H_2O or 1x PBS buffer into the vial of D-LDH standard (Component D) to make 100 U/mL D-LDH standard solution.

PREPARATION OF STANDARD SOLUTION

D-LDH standard

For convenience, use the Serial Dilution Planner:
https://www.aatbio.com/tools/serial-dilution/13808

Add 10 µL of 100 U/mL D-LDH standard solution into 990 µL 1x PBS buffer to generate 1000 mU/mL D-LDH standard solution. Take 1000 mU/mL D-LDH standard solution and perform 1:3 serial dilutions in PBS to get serial dilutions of D-LDH standard (SD7 - SDH1).

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

PREPARATION OF WORKING SOLUTION

1. Add 10 mL of Assay Buffer (Component B) into the bottle of Enzyme Probe (Component A) to have Enzyme Probe mixture.

Note This Enzyme Probe mixture is enough for two 96-well plate.

2. Add 50 µL of 100X NAD stock solution into 5 mL Enzyme Probe mixture and mix well to make D-LDH working solution.

Note This D-LDH working solution is enough for one 96-well plate. It is not stable - make enough for one experiment and use promptly.

SAMPLE EXPERIMENTAL PROTOCOL

Table 1. Layout of D-LDH standards and test samples in a solid black 96-well microplate. SD=D-LDH Standards (SD1 - SD7, 0.3 to 300 mU/mL), BL=Blank Control, TS=Test Samples.

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

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Table 2. Reagent composition for each well.

Well	Volume	Reagent
SD1 - SD7	50 µL	Serial Dilutions (0.3 to 300 mU/mL)
BL	50 µL	Dilution Buffer
TS	50 µL	test sample

1. Prepare D-LDH standards (SD), blank controls (BL), and test samples (TS) 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-LDH working solution to each well of D-LDH standard, blank control, and test samples to make the total D-LDH assay volume of 100 µL/well. For a 384-well plate, add 25 µL of D-LDH 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 at 570 nm).

Note The contents of the plate can also be transferred to a white clear bottom plate and read by absorbance microplate reader at the ratio of A_{575nm}/A_{605nm}. The absorption detection has lower sensitivity compared to fluorescence reading.

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 Dehydrogenase 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

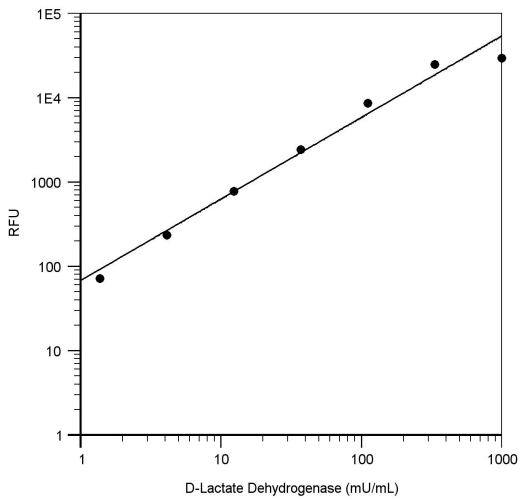

Figure 1. D-LDH dose response was measured with Amplite™ Fluorimetric D-LDH Assay Kit in a 96-well solid black plate using a Gemini (Molecular Devices) microplate reader.

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