Amplite™ Fluorimetric D- Lactate Dehydrogenase Assay Kit

Red Fluorescence

Ordering Information	*Storage Conditions*	*Instrument Platform*
Product Number: 13808 (200 assays)	Keep in freezer Avoid exposure to light	Fluorescence microplate readers

Introduction

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.

AAT Bioquest's Amplite™ Lactate Dehydrogenase Assay Kits (cat#13812 and 13814 for L-lactate dehydrogenase assay, and 13808 and 13809 for D-lactate dehydrogenase assay) provide both fluorescence and absorbance-based method for detecting either L-lactate dehydrogenase (L-LDH) or 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 kit is specific for D-LDH. The fluorescence signal can be read by a fluorescence microplate reader at Ex/Em = 540 nm/590 nm. With this fluorimetric Amplite™ D-lactate Dehydrogenase Assay Kit, we were able to detect as little as 1mU/mL D-LDH in a 100 µL reaction volume. It is robust, and can be readily adapted for a wide variety of applications that require the measurement of D-LDH.

Kit Components

Components	**Amount**
Component A: Enzyme Probe	1 bottle (lyophilized powder)
Component B: Assay Buffer	1 bottle (10 mL)
Component C: NAD	1 vial
Component D: LDH	10U/vial

Assay Protocol for One 96-Well Plate

Brief Summary

Prepare D-lactate dehydrogenase assay mixture (50 µL) → Add D-lactate dehydrogenase standards or test samples (50 µL) → Incubate at room temperature for 30 minutes ~ 2 hours → Monitor fluorescence increase at Ex/Em = 540/590 nm

Note: Thaw one of each kit component at room temperature before starting the experiment.

1. Prepare NAD stock solution (100X):

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

2. Prepare D-LDH stock solution:

Add 100 µL of H_2O or 1xPBS buffer into the vial of D-LDH standard (Component D) to make 100 U/mL D-LDH standard solution.

Note: The unused D-LDH standard stock solution should be divided into single use aliquots and stored at -20°C.

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3. Prepare serial dilutions of D-LDH standard (0 to 300m U/mL):

3.1 Add 10 µL of D-LDH stock solution (from Step 2) into 990 µL 1x PBS buffer to generate 1000 mU/mL D-LDH standard solution.
Note: Diluted D-LDH standard solution is unstable, and should be used within 4 hours.

3.2 Take 200 µL of 1000mU/ml D-LDH standard solution to perform 1:3 serial dilutions to get 300, 100, 30, 10, 3, 1, 0.3and 0 mU/mL serial dilutions of D-LDH standard.

3.3 Add serial dilutions of D-LDH standard and D-LDH containing test samples into a solid black 96-well microplate as described in Tables 1 and 2.

Table 1 Layout of D-LDH standards and test samples in a solid black 96-well microplate

BL	BL	TS	TS	….	….						
D-LDH 1	D-LDH 1	….	….	….	….						
D-LDH 2	D-LDH 2										
D-LDH 3	D-LDH 3										
D-LDH 4	D-LDH 4										
D-LDH 5	D-LDH 5										
D-LDH 6	D-LDH 6										
D-LDH 7	D-LDH 7										

Note: D-LDH=D-LDH Standards, BL=Blank Control, TS=Test Samples.

Table 2 Reagent composition for each well

D-LDH Standard	**Blank Control**	**Test Sample**
Serial Dilutions*: 50 µL	Dilution Buffer : 50 µL	50 µL

**Note: Add the serially diluted D-LDH standards from 0.3 mU/mL to 300 mU/mL into wells from D-LDH1 to D-LDH7 in duplicate.*

4. Prepare D-LDH assay mixture:

4.1 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. The unused enzyme probe mixture should be divided into single use aliquots and stored at -20°C.

4.2 Add 50 µL NAD stock solution (100X) into 5 mL enzyme probe mixture (from Step 4.1), and mix well.
Note: This D-LDH assay mixture is enough for one 96-well plate. It is not stable, make enough for one experiment, and use it promptly.

5. Run D-LDH assay:

5.1 Add 50 µL of D-LDH assay mixture (from Step 4.2) to each well of D-LDH standard, blank control, and test samples (see Step 3.3) to make the total assay volume of 100 µL/well.
Note: For a 384-well plate, add 25 µL of sample and 25 µL assay mixture into each well.

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

5.3 Monitor the fluorescence increase with a fluorescence plate reader at Ex/Em = 530-570/590-600 nm (optimal Ex/Em = 540/590 nm, cut off 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.

Data Analysis

The fluorescence in blank wells (with the dilution buffer only) is used as a control, and is subtracted from the values for those wells with the D-LDH reactions. A typical D-LDH standard curve is shown in Figure 1.
Note: The fluorescence background increases with time, thus it is important to subtract the fluorescence intensity value of the blank wells for each data point.

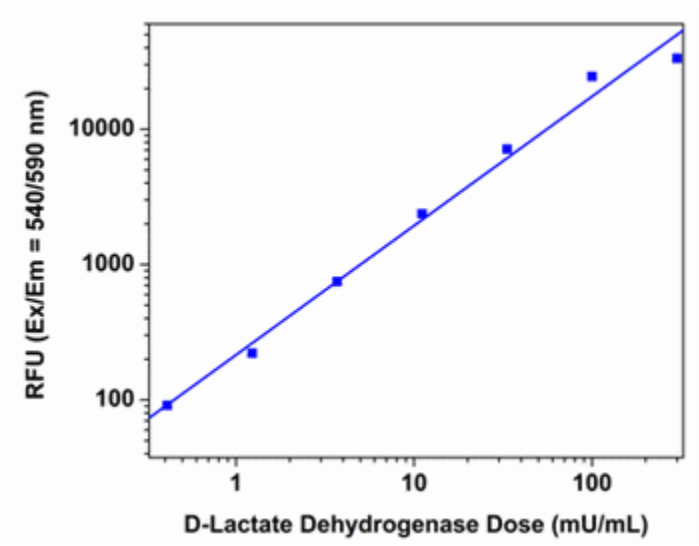

Figure 1. D-LDH dose response was measured with Amplite™ Fluorimetric D-LDH Assay Kit in a 96-well black plate using a Gemini (Molecular Devices) microplate reader. As low as 1mU/mL D-LDH in 100 µL volume can be detected with 30min incubation.

References

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