Amplite™ Colorimetric L- Lactate Dehydrogenase Assay Kit

Ordering Information	*Storage Conditions*	*Instrument Platform*
Product Number: 13813 (200 assays)	Keep in freezer Avoid exposure to light	Absorbance 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-LDH assay, and 13808 and 13809 for D-lactate dehydrogenase assay) provide both fluorescence and absorbance-based method for detecting either L-LDH or 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 L-LDH. The signal can easily read by an absorbance microplate reader at the absorbance ratio of A_{575nm}/A_{605nm}. With the Amplite™ L-Lactate Dehydrogenase Assay Kit, we were able to detect as little as 3mU/mL L-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 L-LDH.

Kit Components

Components	**Amount**
Component A: Enzyme Mix	1 bottle (lyophilized powder)
Component B: Assay Buffer	1 bottle (10 mL)
Component C: NAD	1 vial
Component D: L-Lactate Dehydrogenase	10 U/vial

Assay Protocol for One 96-Well Plate

Brief Summary

Prepare L-lactate dehydrogenase assay mixture (50 µL) → Add L-lactate dehydrogenase standards or test samples (50 µL) → Incubate at room temperature for 30 minutes ~ 2 hours → Monitor absorbance ratio increase at A_{575nm}/A_{605nm}

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 L-LDH stock solution:

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

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

3. Prepare serial dilutions of L-LDH standard (0 to 300mU/mL):

3.1 Add 10 µL of L-LDH stock solution (from Step 2) into 990 µL 1x PBS buffer to generate 1000mU/mL L-LDH standard solution.

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

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3.2 Take 200 µL of 1000mU/ml L-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 L-LDH standard.

3.3 Add serial dilutions of L-LDH standard and L-LDH containing test samples into a white clear bottom 96-well l microplate as described in Tables 1 and 2.

Table 1 Layout of L-LDH standards and test samples in a white clear bottom 96-well microplate

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

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

Table 2 Reagent composition for each well

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

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

4. Prepare L-LDH assay mixture:

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

4.2 Add 100 µL NAD stock solution (100X, from Step 1) into 10 mL enzyme probe mixture (from Step 4.1), and mix well.

Note1: This L-LDH assay mixture is enough for one 96-well plate. It is not stable, and should be used promptly within 2 hours and avoid exposure to light.

Note2: Alternatively, one can make a 50X of L-LDH Enzyme Mixture stock solution by adding 200 µL of H_2O into the bottle of Component A, and then prepare the L-LDH assay mixture by mix the stock solution with assay buffer (Component B) and 100x NAD solution proportionally. Aliquot and store the unused 50X L-LDH Enzyme Mixture stock solution and 100X NAD solution at -20°C, and avoid freeze-thaw cycles.

5. Run L-LDH assay:

5.1 Add 50 µL of L-LDH assay mixture (from Step 4.2) to each well of L-lactate dehydrogenase 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 absorbance ratio increase with an absorbance plate reader at $A_{575nm}/A_{605nm.}$

Data Analysis

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

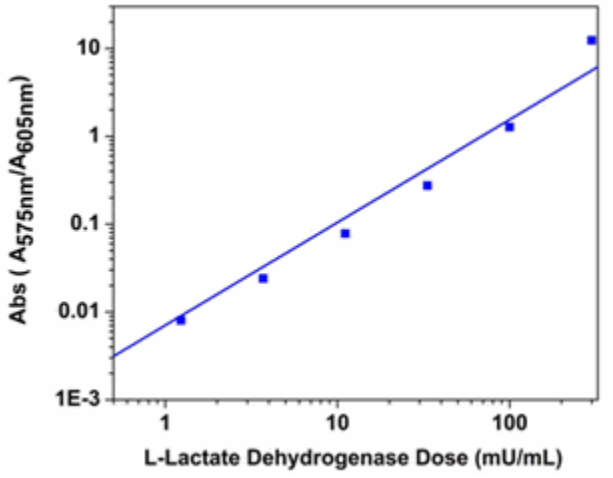

Figure 1. L-LDH dose response was measured with Amplite™ Colorimetric L-Lactate Dehydrogenase Assay Kit in a 96-well white wall/clear bottom plate using a SpectraMax Plus (Molecular Devices) microplate reader. As low as 1mU/mL L-LDH in 100 µL volume can be detected with 30 min incubation.

References

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