

## Amplite™ Colorimetric D- Lactate Dehydrogenase Assay Kit

Ordering Information	Storage Conditions	Instrument Platform
Product Number: 13809 (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-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 chromogenic NADH sensor. This assay kit is specific for D-LDH. The absorption signal can be read by an absorption microplate reader at the absorbance ratio of ~570 nm to ~605 nm ( $A_{575nm}/A_{605nm}$ ). With this colorimetric Amplite™ D-lactate Dehydrogenase Assay Kit, we were able to detect as little as mU/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: D-lactate Dehydrogenase	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 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<sub>2</sub>O into the vial of NAD (Component C) to make 100X NAD stock solution.

#### 2. Prepare D-LDH stock solution:

Add 100 µL of H<sub>2</sub>O 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.*

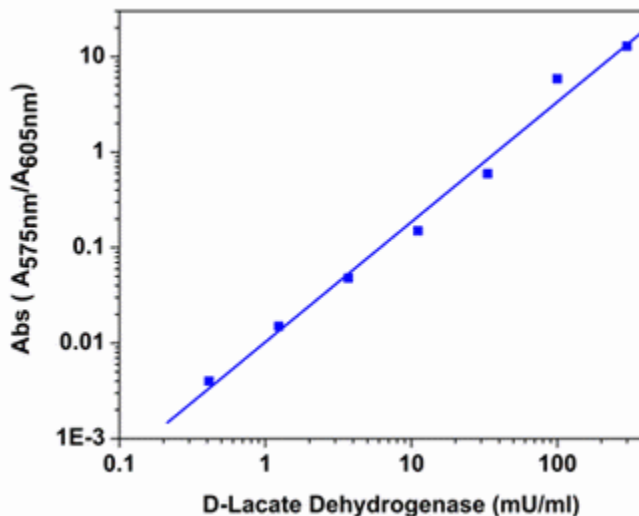
#### 3. Prepare serial dilutions of D-LDH standard (0 to 300 mU/mL):



## 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 D-LDH reactions. A typical D-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.** D-LDH dose response was measured with Amplitude™ Colorimetric D-LDH Assay Kit in a 96-well white wall/clear bottom plate using a SpectraMax Plus (Molecular Devices) microplate reader. As low as 1mU/mL D-LDH in 100  $\mu$ L volume can be detected with 30 min incubation.

## References

1. McLellan A.C., et. al, *Analytical Biochemistry*, 1992, 206(1), 12-16.
2. Beaver W. L., et.al, Improved detection of lactate threshold during exercise using a log-log transformation, *Physiology*, 1985, 59 (6),1936-1940 .
3. Gérson F de Souza, et.al, Lactic acid levels in patients with chronic obstructive pulmonary disease accomplishing unsupported arm exercises, *Chronic Respiratory Disease*, 2010 7:(2) 75-82.
4. Garner H. E., et.al, Lactic acidosis: a factor associated with equine laminitis, *Journal of Animal Science*, 1977, 45:1037-1041.
5. Gladden, L.B. Lactate metabolism: A new paradigm for the third millenium. 2004, *J Physiol* **558**(1) 5-30.
6. Aguirre M., et.al, Lactic acid bacteria and human clinical infection, *Journal of Applied Microbiology*, 1993, 75 (2), 95-107.

**Warning:** This kit is only sold to our authorized distributors or end users. Neither resale nor transfer to a third party is allowed without written permission from AAT Bioquest®, Inc. Chemical analysis of the kit components is strictly prohibited. Please call us at 408-733-1055 or e-mail us at [info@aatbio.com](mailto:info@aatbio.com) if you have any questions.