

Amplite™ Colorimetric D-Lactate Assay Kit

Ordering Information	Storage Conditions	Instrument Platform
Product Number: 13811 (200 assays)	Keep in freezer Avoid exposure to light	Absorbance microplate readers

Introduction

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 yoghurt and cheese, and also in pickled vegetables, and cured meats and fish. The D- and L-lactic acid content 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 chromogenic NADH sensor. The signal can easily read by an absorbance microplate reader at the absorbance ratio of $\sim A_{575nm}/A_{605nm}$. With this Colorimetric Amplite™ D-Lactate Assay Kit, we were able to detect as little as 4 μ M D-lactate in a 100 μ L reaction volume. It is robust, and can be readily adapted for a wide variety of applications that require the measurement of D-lactate.

Kit Components

Components	Amount
Component A: Enzyme Mix	2 bottles (lyophilized powder)
Component B: Assay Buffer	1 bottle (10 mL)
Component C: NAD	1 vial
Component D: D-Lactate Standard	2.25 mg/vial

Assay Protocol for One 96-Well Plate

Brief Summary

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

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

1. Prepare NAD stock solution (100X):

Add 100 μ L of H₂O into the vial of NAD (Component C) to make 100X NAD stock solution.

2. Prepare D-lactate stock solution:

Add 200 μ L of H₂O or 1xPBS buffer into the vial of D-lactate Standard (Component D) to make 100 mM D-lactate standard solution.

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

3. Prepare serial dilutions of D-lactate standard (0 to 1 mM):

- 3.1 Add 10 μ L of D-lactate stock solution (from Step 2) into 990 μ L 1x PBS buffer to generate 1 mM D-lactate standard solution.

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

- 3.2 Take 200 μ L of 1 mM D-lactate standard solution to perform 1:3 serial dilutions to get 300, 100, 30, 10, 3, 1 and 0 μ M serial dilutions of D-lactate standard.

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

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

BL	BL	TS	TS						
Lac1	Lac 1						
Lac 2	Lac 2										
Lac 3	Lac 3										
Lac 4	Lac 4										
Lac 5	Lac 5										
Lac 6	Lac 6										
Lac 7	Lac 7										

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

Table 2 Reagent composition for each well

D-Lactate Standard	Blank Control	Test Sample
Serial Dilutions*: 50 μ L	Dilution Buffer : 50 μ L	50 μ L

**Note: Add the serially diluted D-Lactate standards from 1 μ M to 1 mM into wells from Lac1 to Lac7 in duplicate.*

4. Prepare D-lactate assay mixture:

- 4.1 Add 5 mL of Assay Buffer (Component B) into one bottle of Enzyme Mix (Component A).

- 4.2 Add 50 μ L NAD stock solution (100X, from Step 1) into the bottle of Component A (from Step 4.1), and mix well.

Note1: This D-lactate assay mixture is enough for one 96-well plate. It is unstable, and should be used promptly within 2 hours and avoid exposure to light.

Note2: Alternatively, one can make a 50X of D-Lactate Enzyme Mixture stock solution by adding 100 μ L of H₂O into the bottle of Component A, and then prepare the D-lactate assay mixture by mix the stock solution with assay buffer (Component B) and 100x NAD solution proportionally. Aliquot and store the unused 50X D-Lactate Enzyme Mixture stock solution and 100X NAD solution at -20°C, and avoid freeze-thaw cycles.

5. Run D-lactate assay:

- 5.1 Add 50 μ L of D-lactate assay mixture (from Step 4.2) to each well of D-lactate standard, blank control, and test samples (see Step 3.3) to make the total D-lactate assay volume of 100 μ L/well.

Note: For a 384-well plate, add 25 μ L of sample and 25 μ L of D-lactate 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 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-lactate reactions. A typical D-lactate 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.

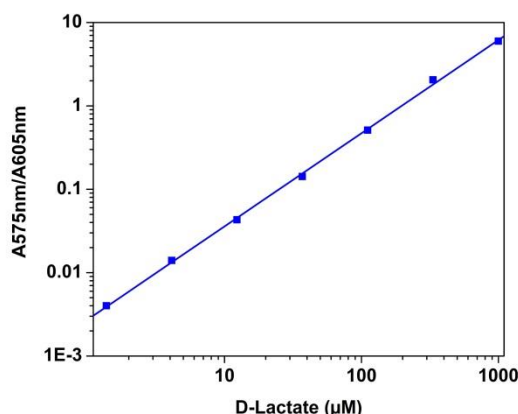


Figure 1. D-lactate dose response was measured with Amplite™ Colorimetric D-lactate Assay Kit in a 96-well white clear bottom plate using a SpectraMax Plus (Molecular Devices) microplate reader. As low as 4 µM D-lactate in 100 µL volume can be detected with 1 hour incubation.

References

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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.

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