Amplite™ Fluorimetric D-Lactate Assay Kit

Red Fluorescence

Ordering Information	*Storage Conditions*	*Instrument Platform*
Product Number: 13810 (200 assays)	Keep in freezer Avoid exposure to light	Fluorescence 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 fluorogenic NADH sensor. The signal can be read by a fluorescence microplate reader at Ex/Em = 540 nm/590 nm. With this Fluorimetric Amplite™ D-Lactate Assay Kit, we were able to detect as little as 1.4 µM D-lactate 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-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 µL) → Add D-lactate standards or test samples (50 µL) → Incubate at room temperature for 30 min ~ 2 hours → Monitor fluorescence increase at Ex/Em = 540/590 nm

Note: Thaw one vial 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-lactate stock solution:

Add 200 µL of H_2O or 1xPBS buffer into the vial of D-lactate Standard (Component D) to make 100 mM D-lacate standard solution.

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

Ordering: sales@aatbio.com; Tel: 800-990-8053 or 408-733-1055; Fax: 408-733-1304
Technical Support: support@aatbio.com; Tel: 408-733-1055

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

3.1 Add 10 µL of D-lactate stock solution (from Step 2) into 990 µL 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 µL of 1 mM D-lactate standard solution to perform 1:3 serial dilutions to get 300, 100, 30, 10, 3, 1 and 0 µM serial dilutions of D-lactate standard.

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

Table 1 Layout of D-lactate standards and test samples in a solid black 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 µL	Dilution Buffer : 50 µL	50 µL

**Note: Add the serially diluted D-Lactate standards from 1 µM 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 µL NAD stock solution (100X, from Step 1) into the bottle of Component A (from Step 4.1), and mix well.
Note: This D-lactate assay mixture is enough for one 96-well plate. The unused D-lactate assay mixture should be divided into single use aliquots and stored at -20°C.

5. Run D-lactate assay:

5.1 Add 50 µL 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 µL/well.
Note: For a 384-well plate, add 25 µL of sample and 25 µL 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 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).

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 fluorescence background increases with time, thus it is important to subtract the fluorescence intensity value of the blank wells for each data point.*

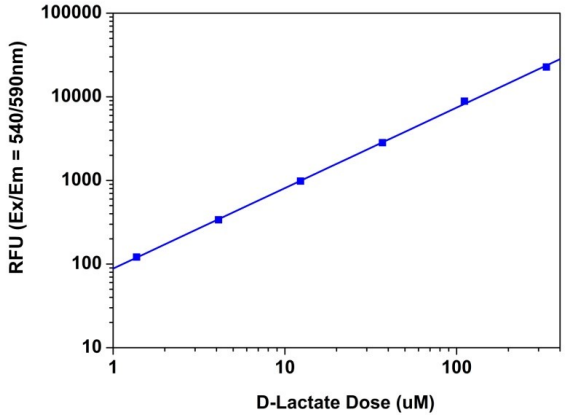

Figure 1. D-lactate dose response was measured with Amplite™ Fluorimetric D-Lactate Assay Kit in a 96-well black plate using a Gemini (Molecular Devices) microplate reader. As low as 1.4 µM D-lactate in 100 µL volume can be detected with 1 hour incubation.

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.