

ab324122 – D-Lactate Dehydrogenase (LDH) Assay Kit (Fluorometric)

A fluorescence-based method for detecting D-lactate dehydrogenase (D-LDH) in biological samples such as serum, plasma, urine, as well as in cell culture samples.

For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit: www.abcam.com/ab324122

Storage and Stability: Store kit at -20°C in the dark immediately upon receipt. Refer to list of materials supplied for storage conditions of individual components.

Materials Supplied

Item	Quantity	Storage Condition
Enzyme Probe	1 bottle (lyophilized powder)	-20°C (Store in the dark)
Assay Buffer	1 bottle (10 mL)	-20°C
NAD	1 vial	-20°C (Store in the dark)
LDH	10 U/vial	-20°C (Store in the dark)

Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully utilize this assay:

- Fluorescence microplate reader (550/590 nm)
- Solid black plates
- H₂O
- 1 x PBS buffer

Protocol Summary

1. Prepare D-lactate Dehydrogenase working solution (50 µL).
2. Add D-lactate Dehydrogenase standards or test samples (50 µL).
3. Incubate at room temperature for 30 minutes - 2 hours.
4. Monitor fluorescence increase at Ex/Em = 540/590 nm (Cutoff = 570nm).

IMPORTANT: Thaw all kit components at room temperature before starting the experiment.

Preparation of Stock Solutions

Unless otherwise noted, all unused stock solutions should be divided into single-use aliquots and stored at -20 °C after preparation. Avoid repeated freeze-thaw cycles.

NAD stock solution (100X)

1. Add 100 µL of H₂O into the vial of NAD to make 100X NAD stock solution.

D-LDH standard solution (100 U/mL)

1. Add 100 µL of H₂O or 1x PBS buffer into the vial of D-LDH standard to make 100 U/mL D-LDH standard solution.

Preparation of Standard Solution

D-LDH Standard solution

1. Add 10 µL of 100 U/mL D-LDH standard solution into 990 µL 1x PBS buffer to generate 1000 mU/mL D-LDH standard solution. Take 1000 mU/mL D-LDH standard solution and perform 1:3 serial dilutions in PBS to get serial dilutions of D-LDH standard (SD7 - SDH1).

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

Preparation of Working Solutions

Enzyme Probe Mixture

1. Add 10 mL of Assay Buffer into the bottle of Enzyme Probe to have Enzyme Probe mixture.

Note: This Enzyme Probe mixture is enough for two 96-well plate.

D-LDH working solution

1. Add 50 µL of 100X NAD stock solution into 5 mL Enzyme Probe mixture and mix well to make D-LDH working solution.

Note: This D-LDH working solution is enough for one 96-well plate. It is not stable - make enough for one experiment and use promptly.

Experimental Protocol

BL	BL	TS	TS
SD1	SD1
SD2	SD2
SD3	SD3		
SD4	SD4		
SD5	SD5		
SD6	SD6		
SD7	SD7		

Table 1. Layout of D-LDH standards and test samples in a solid black 96-well microplate. SD=D-LDH Standards (SD1 - SD7, 0.3 to 300 mU/mL), BL=Blank Control, TS=Test Samples.

Well	Volume	Reagent
SD1 – SD7	50 µL	Serial Dilutions (0.3 to 300 mU/mL)
BL	50 µL	Dilution Buffer
TS	50 µL	Test Sample

Table 2. Reagent composition for each well of a 96-well microplate.

1. Prepare D-LDH standards (SD), blank controls (BL), and test samples (TS) according to the layout provided in Tables 1 and 2. For a 384-well plate, use 25 µL of reagent per well instead of 50 µL.
2. Add 50 µL of D-LDH working solution to each well of D-LDH standard, blank control, and test samples to make the total D-LDH assay volume of 100 µL/well. For a 384-well plate,

add 25 μ L of D-LDH working solution into each well instead, for a total volume of 50 μ L/well.

3. Incubate the reaction at room temperature for 30 minutes to 2 hours, protected from light.
4. Monitor the fluorescence increase with a fluorescence plate reader at:
Excitation = 530 - 570 nm
Emission = 590 - 600 nm (optimal Ex/Em = 540/590 nm, Cutoff at 570 nm)

Data Analysis

The reading (RFU) obtained from the blank standard well is used as a negative control. Subtract this value from the other standards' readings to obtain the base-line corrected values. Then, plot the standards' readings to obtain a standard curve and equation.

For technical support contact information, visit: www.abcam.com/contactus

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