ab324123 – D-Lactate Dehydrogenase (LDH) Assay Kit (Colorimetric)

An absorption-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/ab324123

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)
D-lactate Dehydrogenase	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:

- Absorbance microplate reader (575/605 nm)
- Clear bottom plates
- H_2O
- 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 absorbance ratio increase at A_{575nm}/A_{605nm}.

IMPORTANT: Thaw one of each kit component 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 H_2O into the vial of NAD to make 100X NAD stock solution.

D-LDH standard solution (100 U/mL)

1. Add 100 µL of H_2O 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. It is unstable at room temperature and should be used promptly within 2 hours and avoid exposure to light. Alternatively, one can make a 50X of D-LDH Enzyme Mixture stock solution by adding 200 µL of H_2O into the bottle of Enzyme Probe, and then prepare the D-LDH working solution by mix the stock solution with assay buffer and 100x NAD solution proportionally.*

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 clear bottom 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 µL of D-LDH working solution into each well instead, for a total volume of 50 µL/well.
3. Incubate the reaction at room temperature for 30 minutes to 2 hours, protected from light.
4. Monitor the absorbance ratio increase with an absorbance plate reader at A_{575nm}/A_{605nm}.

Data Analysis

The reading (Abs 575/ Abs 605) 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

Version 1 | 2025-05-06