CREATIVE BIOMART®
Assay Kit

L-Lactate Dehydrogenase Assay Kit

Product Information

Common Name

LDH

Cat.No. Kit-2087

Product Overview

Lactate Dehydrogenase Assay Kits provide both fluorescence and absorbance-based method for detecting either L-LDH or 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 fluorogenic NADH sensor. This assay kit is specific for L-LDH. The signal can easily read by an absorbance microplate reader at ~575 nm or at the absorbance ratio of A575nm/A605nm to increase assay sensitivity. With the L-Lactate Dehydrogenase Assay Kit, we were able to detect as little as 3mU/mL LLDH in a 100 µL reaction volume.

Description

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.

Storage

Freeze (<-15 °C), Minimize light exposure

Kit Components

Component A: Enzyme Probe: 1 bottle (lyophilized powder), Freeze (<-15 °C), Dessicated, Avoid Light
Component B: Assay Buffer: 1 bottle (10 mL), Freeze (<-15 °C)
Component C: NAD: 1 vial, Freeze (<-15 °C), Dessicated, Avoid Light
Component D: L-lactate Dehydrogenase: 10U/vial, Freeze (<-15 °C), Dessicated, Avoid Light

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Detection method 575/605 nm

Preparation

Preparation of stock solution
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.
1. NAD stock solution (100X):
Add 100 µL of H2O into the vial of NAD (Component C) to make 100X NAD stock solution.
2. L-Lactate Dehydrogenase (L-LDH) standard solution (100 U/mL):
Add 100 µL of H2O or 1x PBS buffer into the vial of L-Lactate Dehydrogenase (Component D) to make 100 U/mL L-LDH standard solution.

Preparation of standard solution
L-LDH standard:
Add 10 µL of 100 U/mL L-LDH standard solution into 990 µL 1x PBS buffer to generate 1000 mU/mL L-LDH standard solution. Take 1000 mU/mL L-LDH standard solution and perform 1:3 serial dilutions in 1x PBS buffer to get serially diluted L-LDH standards (SD7 - SD1).
Note: Diluted L-LDH standard solution is unstable, and should be used within 4 hours.

Preparation of working solution
1. Add 10 mL of Assay Buffer (Component B) into the bottle of Enzyme Mix (Component A), and mix well.
2. Add 100 µL of 100X NAD stock solution into the bottle of Component A+B and mix well to make L-LDH working solution.
Note: This L-LDH working solution is enough for one 96-well plate. It is unstable and should be used promptly within 2 hours. Avoid exposure to light.
Note: Alternatively, one can make a 50X of L-LDH Enzyme Mix stock solution by adding 200 µL of H2O into the bottle of Enzyme Mix (Component A), and then prepare the L-LDH working solution by mix the stock solution with Assay Buffer (Component B) and 100X NAD stock solution proportionally.

Assay Protocol

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1. Prepare L-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.
Table 1. Layout of L-LDH standards and test samples in a white clear bottom 96-well microplate. SD= L-LDH Standards (SD1 - SD7, 0.4 to 300 mU/mL), BL=Blank Control, TS=Test Samples.
BL BL TS TS
SD1 SD1 ... ...
SD2 SD2 ... ...
SD3 SD3
SD4 SD4
SD5 SD5
SD6 SD6
SD7 SD7

Table 2. Reagent composition for each well.
Well Volume Reagent
SD1 - SD7 50 µL Serial Dilutions (0.4 to 300 mU/mL)
BL 50 µL Dilution Buffer
TS 50 µL test sample

2. Add 50 µL of L-LDH working solution to each well of L-LDH standard, blank control, and test samples to make the total L-LDH assay volume of 100 µL/well. For a 384-well plate, add 25 µL of L-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 A575nm/A605nm.

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. This equation can be used to calculate L-Lactate Dehydrogenase samples.

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