MANUAL

RayBio® Lactate Dehydrogenase Activity Assay Kit (Colorimetric)

Catalog #: MA-LDH

RayBiotech
Empowering your proteomics

ISO 13485:2016

Introduction

Lactate dehydrogenase (LDH) is a crucial enzyme in the anaerobic metabolic pathway, catalyzing the reversible conversion of lactate to pyruvate while reducing NAD+ to NADH and vice versa. LDH is ubiquitously present in various body tissues. Elevated LDH levels in the bloodstream can result from diverse conditions, including liver disease, anemia, heart attack, bone fracture, muscle trauma, cancer, and infection. LDH is also a general marker of tissue turnover, reflecting normal metabolic processes.

The RayBio® Lactate Dehydrogenase Activity Assay Kit offers a user-friendly, reliable, and highly sensitive method for quantifying LDH activity in plasma, serum, cell lysates, and other biological fluid samples. In this assay, LDH facilitates the oxidation of lactate into pyruvate while concurrently reducing NAD to NADH. The rate of NAD reduction can be quantified by monitoring the increase in absorbance at 340nm. This rate of absorbance change is directly proportional to the LDH activity within the sample.

Storage

The entire kit may be stored at 2–8 °C for up to 6 months from the date of shipment. For prepared reagent storage, see table below.

Component	Size / Description	Storage After Preparation
Microplate (Item A)	A 96-well (12 strips x 8 wells) plate	RT*
Sample Buffer	10 ml	2–8 °C
Assay Buffer	20 ml	2–8 °C
Enzyme Mix Solution	4 ml	2–8 °C
Lactate Dehydrogenase (LDH) Positive Control	1 Vial (10 µl)	2–8 °C

RT = room temperature

**Return unused wells to the pouch containing desiccant pack, reseal along entire edge.*

Additional Materials Required

1. Microplate reader capable of measuring absorbance at 340 nm at 37°C
2. Precision pipettes to deliver 2 µl to 1 ml volumes
3. Multi-channel pipettes to deliver 20 µl to 200 µl volumes
4. Tubes to prepare sample dilutions
5. Incubator at 37°C
6. 50 ml conical tubes

Sample Tips and General Considerations

NOTE: Optimal methods of sample preparation will need to be determined by each researcher empirically based on researched literature and knowledge of the samples.

- If not using fresh samples, freeze samples as soon as possible after collection.
- Avoid multiple freeze-thaw cycles. If possible, sub-aliquot samples prior to initial storage.
- It is strongly recommended to add a protease inhibitor cocktail to cell and tissue lysate samples.
- Avoid sonication of 1 ml or less as this can quickly heat and denature proteins.
- Most samples will not need to be concentrated. If concentration is required, a spin column concentrator with a chilled centrifuge is recommended.

1. Cell lysates can be prepared as follows:

For attached cells, remove supernatant from cell culture, wash cells twice with cold 1X PBS (for suspension cells, pellet the cells by spinning down the cells at 1,000 x g for 10 min) making sure to remove any remaining PBS before adding lysis buffer. Solubilize the cells at $2x10^7$ cells/ml in lysis buffer containing protease inhibitors. Pipette up and down to resuspend cells and rock the lysates gently at 2–8 °C for 30 minutes. Transfer extracts to microfuge tubes and centrifuge at 14,000 x g for 10 minutes.
It is recommended that sample protein concentrations should be determined using a total protein assay. Lysates should be used immediately or aliquot and stored at -70 °C. Thawed lysates should be kept on ice prior to use.
General tips for preparing lysate samples can be viewed on the online Resources page of the website: https://www.raybiotech.com/tips-on-sample-preparation/

2. Plasma samples:

Collect blood with an anticoagulant such as citrate, EDTA or oxalate and mix by inversion. Centrifuge the blood at 1000 x g at 4°C for 10 minutes. Collect plasma supernatant without disturbing the white buffy layer. Sample should be tested immediately or frozen at -80°C for storage. No dilution/Neat is recommended for a typical plasma sample.

3. Serum samples:

Collect blood in a tube with no anticoagulant. Allow the blood to clot at room temperature for 30 minutes. Centrifuge at 2500 x g for 20 minutes. Remove the yellow serum supernatant without disturbing the white buffy layer. Samples should be tested immediately or frozen at -80°C for storage. No dilution/Neat is recommended for a typical serum sample.

NOTE:
If the calculated LDH activity of the sample is higher than 1000 U/L, dilute the sample in Sample Buffer and repeat the assay.

Reagent Preparation

A. Working Solution

For one 96-well plate, mix 20ml of Enzyme Mix Solution and 4ml of Assay Buffer to prepare the Working Solution. Mix well. Protect from light. Discard remaining reagent after completion of testing.

B. Positive Control

Lactate Dehydrogenase (LDH) Positive Control: pipette 1 µL of LDH Positive Control into 49 µL Sample Buffer to create a positive control stock, mix well. Then pipette 5 µL of the positive control stock into 295 µL Sample Buffer to prepare the positive control sample. Mix well.

Assay Procedure

Positive control, sample and Sample Buffer (used as a blank) should be assayed in duplicate or triplicate. A freshly prepared positive control should be used each time the assay is performed.

1. Set up a microplate reader or a microplate incubator at 37°C.
2. Prepare Working Solution (See Reagent Preparation, section A), and incubate it at 37°C for five minutes.
3. Prepare Positive Control (See Reagent Preparation, section B).
4. Pipette 10 µL of sample, Sample Buffer (as the blank) and Positive Control into each well of the 96-well plate.
5. Transfer 200 µL of pre-warmed Working Solution into each well (it is recommended to use a multi-channel pipette), mix and incubate at 37°C for 30 seconds.
6. After 30 seconds, read and record absorbance at 340nm (A1 Reading). Continue Incubating at 37°C for one minute.
7. After exactly one minute, read and record absorbance at 340nm (A2 Reading).

Calculation of Results

Calculate the mean absorbance for each set of duplicate sample, Positive Control, and Sample Buffer blank. Subtract the average Sample Buffer blank optical density for each time point.

$$A1' = A1 - A1_{Blank},\ A2' = A2 - A2_{Blank}$$

One international Unit (U/L) is defined as the amount of enzyme that catalyzes the transformation of one micromole of substrate per minute.

LDH activity (U/L)= $\frac{(A2'-A1')\times 210\mu l\times 1000}{3.66mM^{-1}\times 10\mu l}\times sample\ dilution$

Where: *A2'-A1'* = Change in absorbance

1000 = Conversion of U/ml to U/L

$3.66mM^{-1}$ is the adjusted extinction coefficient for NADH at 340nm with the current path length of the solution in a 96-well plate.

*LDH activity SI Units (nkat/L) = LDH activity (U/L)*16.67*

Note: If the calculated LDH activity of the sample is higher than 1000 U/L, dilute the sample in Sample Buffer and repeat the assay.

A. Typical Data

	A2'-A1'	LDH activity (U/L)
Serum	0.0095	54.51
Plasma (EDTA)	0.0065	37.30
Plasma (Citrate)	0.003	17.21
Plasma (Heparin)	0.008	45.90
HepG2 cell lysate	0.0005	2.87
LDH Positive Control	0.0765	438.93
Blank	0	0

B. Linear Range

Up to 1000 U/L LDH activity

C. Reproducibility

Intra-assay Precision (Precision within an assay):
To assess intra-assay precision, 16 wells per sample (total of 4 samples) were tested on a single plate. The intra-assay coefficient of variation was found to be 6.7%.

Inter-assay Precision (Precision between assays):
To assess inter-assay precision, 4 samples were tested in separate assays (n=4). The inter-assay coefficient of variation was found to be 8.5%.

This product is for research use only.