Lactate Dehydrogenase Activity Assay Kit (WST-8)

Introduction

Lactate dehydrogenase (LDH) is a key metabolic enzyme widely present in living cells, which can catalyze the reversible conversion of lactate to pyruvate, as it converts NAD^+ to NADH. When cells are damaged, LDH is released into the blood or body fluids, so it is often considered an important biomarker for evaluating tissue damage (e.g., myocardial infarction, hepatitis, tumors). The detection of LDH activity is of great significance for clinical disease diagnosis.

This kit provides a quickly, convenient and sensitive assay to detect LDH activity in serum, tissue, cell and other samples. In this assay, LDH catalyzes lactate to produce pyruvate and reduces NAD^+ to NADH, which can react with WST-8 to produce orange-yellow formazan. Formazan has a specific absorption at 450 nm and its absorbance is proportional with LDH enzyme activity.

Components and Storage

Components \ Size	100 Assays	500 Assays	Storage
LDH Assay Buffer	16 mL	80 mL	-20°C
Substrate Mix	1 vial	5 x 1 vial	-20°C away from light
NADH Standard	1 vial	1 vial	-20°C
Chromogenic Agent	0.22 mL	1.1 mL	-20°C away from light
Stop Solution	6 mL	30 mL	-20°C
Shipping: Blue ice	Shelf life: 1 year		

Protocol

1. Preparation before the experiment

   1) NADH Standard Preparation: Dissolve a vial of NADH Standard with 0.58 mL of ddH_2O to make a 5 mM NADH standard. For the first use, aliquot and store at -20°C. Use within one month. Place on ice when using.

   2) Substrate Mix Preparation: Dissolve a vial of Substrate Mix with 0.22 mL of ddH_2O to make the Substrate Mix solution. For the first use, aliquot and store at -20°C. Use within one month. Place on ice when using.

2. Sample preparation

1) Cell sample

i. Collect 1-2 x 10^6 cells, wash cells once with pre-chilled PBS.

ii. Add 400 µL of pre-chilled LDH Assay Buffer, pipette several times, and lyse cells on ice for 10 min.

iii. Centrifuge at 10,000 g at 4°C for 15 min.

iv. Transfer the supernatant to a new tube and put it on ice. At this time, measure protein concentration.

***Note:** BCA Protein Assay Kit (K4101) can be used to detect protein concentration.

2) Tissue sample

i. Weigh 100 mg of tissue, wash once with pre-chilled PBS.

ii. Homogenize tissue on ice with 500 µL of pre-pre-chilled LDH Assay Buffer.

iii. Centrifuge at 10,000 g at 4°C for 15 min.

iv. Transfer the supernatant to a new tube and put it on ice. At this time, measure protein concentration.

***Note:** BCA Protein Assay Kit (K4101) can be used to detect protein concentration.

3) Liquid samples such as serum and urine

i. Use directly. To ensure the reading will fall within the standard values, it is suggested to perform several dilutions of the samples. Dilution can be done with LDH Assay Buffer by referring to the table below.

sample	Recommended Dilution factor
Human serum	10-20
Dog serum	10-20
Mouse serum	50-100
Cynomolgus monkey serum	10-20
10% rat spleen tissue homogenate	150-250
10% mouse liver tissue homogenate	250-350
10% rat kidney tissue homogenate	250-350
10% rat lung tissue homogenate	250-350

3. Standards Preparation: Dilute the 5 mM NADH standard according to the table below.

Standard #	1	2	3	4	5	6	7	8
5 mM NADH Standard (µL)	0	2	4	6	8	10	12	16

LDH Assay buffer (μL)	200	198	196	194	192	190	188	184
Standard concentration (μmol/L)	0	50	100	150	200	250	300	400

4. LDH activity detection

1) Set up reaction wells.

Sample well	Take 2-50 μL of sample and adjust the volume to 50 μL with the LDH Assay Buffer.
Standard well	Take 50 μL of the standard at different concentrations.

2) Each well requires 50 μL of the reaction working solution as shown in the table below.

Assay Buffer (μL)	46
Substrate Mix (μL)	2
Chromogenic Agent (μL)	2
Total (μL)	50

3) Add 50 μL of the reaction working solution to each well and incubate at 37°C for 10 min.

4) After incubation, add 50 μL of Stop Solution to each well.

5) Measure absorbance at 450 nm by a microplate reader.

5. Analysis of results

Plot the standard curve: y=ax+b

Serum, plasma and other liquid samples:

Unit definition: One unit of LDH activity is the amount of enzymatic activity that catalyzes the substrate to produce 1 μmoL of NADH per minute per liter of liquid sample at 37°C.

LDH enzyme activity (U/L) = (ΔA450 - b) ÷ a ÷ T × D

Cell and tissue samples:

Unit definition: One unit of LDH activity is the amount of enzymatic activity that catalyzes the substrate to produce 1 μmoL of NADH per minute per g tissue protein or cell protein at 37°C.

LDH enzyme activity (U/gprot) = (ΔA450 - b) ÷ a ÷ T × D ÷ Cpr

***Note:**

y: Standard A450 - Blank A450, blank is the standard #1

x: Standard concentration

ΔA450: Sample A450 - Blank A450

T: Reaction time, 10 min

D: The sample dilution factor before adding to the reaction system

Cpr: sample protein concentration before addition to the reaction system, gprot/L

Notes

1. If the sample concentration is too high or too low, dilute or concentrate the sample appropriately.
2. For your safety and health, please wear lab coats and gloves during the experiment.
3. For research use only. Not to be used in clinical diagnostic or clinical trials.

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