ABS_Bio™ Lactate Dehydrogenase Activity Detection Kit

(Cat# K107-100; 100 assays; store kit at -20℃)

Introduction

Lactate dehydrogenase (LDH) is an oxidoreductase enzyme that catalysis the interconversion of pyruvate and lactate. LDH is an enzyme that is found in almost all of the body's cells (as well as in bacteria) and is released from cells into the fluid portion of blood (serum or plasma) when cells are damaged or destroyed. Thus, the blood level of LDH is a general indicator of tissue and cellular damage. The level of LDH may also rise in the presence of liver diseases, anemia, leukemia, cancer and severe infections.

The ABS_Bio™ Lactate Dehydrogenase Activity Detection Kit provides a simple, sensitive, one-step high-throughput adaptable colorimetric assay to detect LDH in various samples. In this assay, Lactate dehydrogenase converts lactate and NAD^+ into pyruvate and NADH, NADH reduces the tetrazolium salt WST-1 to formazan through the redox activity. The reduced formazan is soluble and its absorption at 440 nm is proportional to the amount of LDH activity in the sample. The kit is supplied with sufficient reagents for 100 tests in 96-well plate assay, linear detection range of 0.03-200 U/L with only 20 µL sample volume.

Kit Components (100 tests)

Assay Buffer:	15 mL	Developer:	0.22 mL	Diaphorase:	0.12 mL	NADH Standard:	1 vial Lyophilized
Substrate:	0.25 mL						

Storage and Handling: Store kit at -20℃. Shelf Life: 6 months after receipt. Warm up Reagents to room temperature before use.

Protocol

1. Sample preparation

Biological samples such as serum, plasma, urine should be directly assay. Cells or tissues can be homogenized in 4 volumes of the assay buffer or PBS. Centrifuge the samples at 10,000 ×g for 10 minutes at 4℃ to remove insoluble material. Samples should be serially diluted to make sure the readings are within the detection limitation range. For samples with potential endogenous enzyme activity or NADH (NADPH) background, should set up no-substrate sample blank control.
Transfer 20 µL sample into the 96-well flat bottom plate in duplicate.

2. Standard Preparation

Add 100 µL dH_2O into NADH standard tube to generate 5 mM NADH solution. Transfer 20 µL 5 mM NADH into 180 µL dH_2O to generate a 500 µM NADH standard, then following the table to generate 500, 300, 150, 50 and 0 µM NADH standard.

NADH std(µL)	dH_2O(µL)	final NADH concentration (µM)
50	0	500
30	20	300
15	35	150
5	45	50
0	50	0

Transfer 20 µL of appropriate standards into the 96-well plate in duplicate. The blank control containing dH_2O only.

3. Reaction

Prepare enough working reagent by mixing 80 µL assay buffer, 2 µL developer, 1 µL diaphorase, 2 µL substrate for each reaction(For sample background control, mixing 78 µL assay buffer, 2 µL developer, 1 µL diaphorase, no substrate).
The assay is based on an enzyme-catalyzed kinetic reaction, using a multi-channel pipettor is recommended to transfer 80 µL prepared working reagent into each reaction well quickly. Tap plate to mix well.

4. Measurement

Read the optical density at 440 nm (430-460nm). Because the assay is continuous (not terminated if don't add stop solution), we suggest to measure absorbance at multiple time points (0 or 20 min) to follow the kinetics of the reactions at room temperature or 37℃ (incubation times will depend on the LDH activity in the samples).

5. Calculation

Average the duplicate OD440 nm reading for standard and sample. Subtract the average OD of the blank (OD_{blank}) from the average OD of the standards ($OD_{20\ std}$) and sample ($OD_{20\ sample}$) at 20 minutes, then plot the standard result ($\Delta OD_{\ std} = OD_{20\ std} - OD_{blank}$) versus the NADH concentration to get NADH standard curve. Calculate sample results ($\Delta OD_{\ sample} = OD_{20\ sample} - OD_{0\ sample}$), and determine sample's LDH generated NADH (µM) from NADH standard curve.

Calculate the LDH activity of samples as equation.

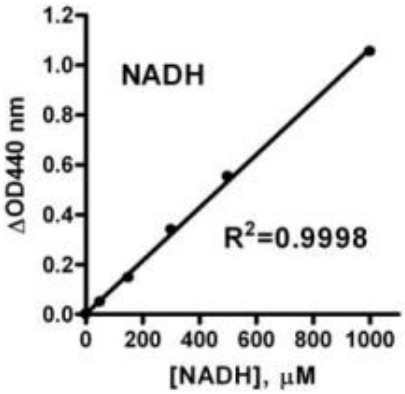
[LDH]= µM NADH/ (*t* * *v*) * *n* (U/mL)

***t* is the reaction time (20 min), *v* is the sample volume (0.02 mL), *n* is the sample dilution factor.**

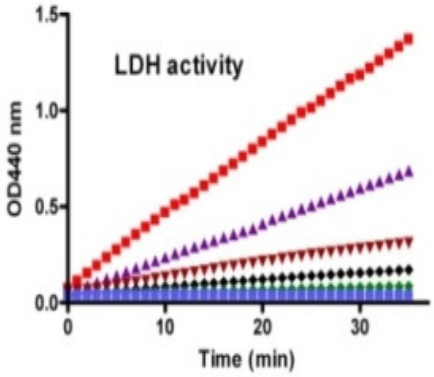
Unit Definition: One unit is the amount of LDH that will generate 1.0 µM of NADH per min at pH 8.5 at 25℃.

Note: We recommend to choose the period of linear range within the standard curve to calculate the LDH activity of the samples. If sample LDH activity over the linear range, either use a shorter reaction time or dilute samples in water and repeat the assay. For samples with lower LDH activity, the incubation time can be extended to 2 hours or incubate at 37℃.

Typical Standard Curve



NADH standard in 96 wells-plate assay (colorimetric).



Series dilute FBS sample in 96 wells-plate assay

Always run your own standard curves for calculation of results.

Sensitivity and Limit of Detection

This reaction has been used to detect as little as 0.02 U/L in a 100 µL volume. The Limit of Detection was determined as 0.03, and linear detection range up to 200 U/L LDH in 96-well plate assay. Samples with values above 200 U/L should be dilute with dH_2O or assay buffer, re-assayed, and multiply results by dilution factor.

Interferences

Culture media contain phenol red in DMEM (15mg/L) and RPMI 1640 (5mg/L) were tested in the assay for interference in assay buffer. No significant change in the measured lactate level was observed.

Culture media contain 10% FBS will increase the signal by potential endogenous enzyme activity, culture media as blank control should be run parallel.

References

Powers, JL. et al. 2007, Biochemistry & Molecular Biology Education. 35:287-292
Baba, C. et al. 2005, Antiviral Chemistry & Chemotherapy. 16:33-39
Legrand, C. et al. 1992, J. Biotechnology. 25:231-243

Related Products:

ALP Colorimetric Detection Kit (#K109-200)
ALP Fluorimetric Detection Kit (#K110-200)
ALP Substrate Solution (#C8040)
BCIP/NBT Substrate system (#C8041)
Creatinine Detection Kit (#K148-250)
Glucose Detection Kit (#K188-100)
Glutathione Detection Kit (#K140-100)
Glutathione Peroxidase Detection Kit (#K143-100)
Glutathione Reductase Detection Kit (#K146-100)
HRP Fluorimetric Detection Kit (#K210-100)
HRP Substrate system (#C8042)
Protein Detection Kit (#K138-200)
Lactate Fluorimetric Detection Kit (#K208-100)
Heme Detection Kit (#K169-200)
Alcohol/Ethanol Detection Kit (#K105-100)
NAD/NADH Detection Kit (#K120-100)
NADP/NADPH Detection Kit (#K123-100)
Nitric Oxide Detection Kit (#K116-100)
Phosphatase Detection Kit (#K196-500)
Urea Detection Kit (#K158-200)
pNPP substrate solution (#C8071)
TBARS Detection Kit (#K145-100)
Peroxidase Activity Detection Kit(#K126-200)
Hemoglobin Detection Kit (#K168-200)
Phosphate Detection Kit (#K198-500)
Alcohol Dehydrogenase Activity Detection Kit(#K113-100)