Lactate Dehydrogenase (LDH) Assay Kit (Colorimetric)

LS-K306-100 (100 Tests) • **Store at -20°C**

Introduction

LACTATE DEHYDROGENASE (LDH) is an oxidoreductase which catalyzes the interconversion of lactate and pyruvate. When disease or injury affects tissues containing LDH, the cells release LDH into the bloodstream, where it is identified in higher than normal levels. Therefore, LDH is most often measured to evaluate the presence of tissue or cell damage. The non-radioactive colorimetric LDH assay is based on the reduction of the tetrazolium salt MTT in a NADH-coupled enzymatic reaction to a reduced form of MTT which exhibits an absorption maximum at 565 nm. The intensity of the purple color formed is directly proportional to the enzyme activity.

Key Features

- High sensitivity and wide linear range. Use 3 µL serum or plasma sample. The detection limit is 2 U/L, linear up to 200 U/L.
- Homogeneous and simple procedure. Simple "mix-and-measure" procedure allows reliable quantitation of LDH activity within 30 minutes.
- Robust and amenable to HTS. All reagents are compatible with high-throughput liquid handling instruments.

Applications

- Direct Assays: LDH activity in serum, plasma and other sources.
- Characterization and Quality Control for LDH production.
- Drug Discovery: screen and evaluation of LDH modulators.

Components

Component	K306-100
	100 Tests
Substrate Buffer	20 mL
NAD Solution	1 mL
MTT Solution	1.5 mL
Diaphorase	120 µL
Calibrator	1.5 mL

Materials Not Supplied

Pipetting devices and accessories (e.g. multi-channel pipettor), clear flat-bottom 96-well plates (e.g. VWR cat# 82050-760) or cuvettes and plate reader or spectrophotometer capable of measuring OD 565 nm.

Storage

The kit is shipped at ambient temperature. Store all components at -20°C upon receiving. Shelf life: 6 months after receipt.

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Assay Procedure

This assay is based on a kinetic reaction. To ensure identical incubation time, addition of Working Reagent to samples should be quick and mixing should be brief but thorough. Use of a multi-channel pipettor is recommended. Assays can be executed at room temperature or 30°C.

Sample Preparation

Serum and plasma are assayed directly.

Tissue: prior to dissection, rinse tissue in phosphate buffered saline (pH 7.4) to remove blood. Homogenize tissue in 5 mL buffer containing 100 mM potassium phosphate (pH 7.0) and 2 mM EDTA, per gram tissue. Centrifuge at 10,000 x g for 15 min at 4°C. Remove supernatant for assay.

Cell Lysate: collect cells by centrifugation at 2,000 x g for 5 min at 4°C. For adherent cells, do not harvest cells using proteolytic enzymes; rather use a rubber policeman. Homogenize or sonicate cells in an appropriate volume of cold buffer containing 100 mM potassium phosphate (pH 7.0) and 2 mM EDTA. Centrifuge at 10,000 x g for 15 min at 4°C. Remove supernatant for assay.

All samples can be stored at –20 to –80°C for at least one month.

Reagent Preparation

Equilibrate reagents to desired reaction temperature (e.g. 25°C or 37°C). Briefly centrifuge reagent tubes before use.

Prepare sufficient Working Reagent (WR) for all sample wells by mixing, for each well: 14 µL MTT Solution, 8 µL NAD Solution, 1 µL Diaphorase and 175 µL Substrate Buffer. Fresh reconstitution is recommended.

Procedure

1. Transfer 200 µL H_2O (OD_{H2O}) and 200 µL Calibrator (OD_{CAL}) solution into separate wells of a clear flat bottom 96-well plate.
2. Transfer 10 µL of each sample into separate wells and then add 190 µL WR to each sample well. Tap plate briefly to mix.
3. Read OD_{565nm} immediately (OD_{S0}), and again after 25 min (OD_{S25}) on a plate reader.

Calculations

LDH activity can then be calculated as follows:

$$\text{LDH Activity} = \frac{OD_{S25} - OD_{S0}}{\varepsilon_{mtt} \bullet l} \times \frac{\text{Reaction Vol (µL)}}{\text{Time} \bullet \text{Sample Vol (µL)}} \times n$$

$$= 43.68 \times \frac{OD_{S25} - OD_{S0}}{OD_{CAL} - OD_{H20}} \times n \quad \text{(IU/L)}$$

where OD_{S25} and OD_{S0} are OD_{565nm} values of sample at 25 min and 0 min. ε_{mtt} is the molar absorption coefficient of reduced MTT. l is the light path length which is calculated from the calibrator. OD_{CAL} and OD_{H20} are OD_{565nm} values of the Calibrator and water. Reaction Vol and Sample Vol are 200 µL and 10 µL, respectively. n is the dilution factor.

Note: if sample LDH activity exceeds 200 IU/L, dilute samples in water and repeat the assay.

Unit definition: 1 Unit (IU) of LDH will catalyze the conversion of 1 µmole of lactate to pyruvate per min at pH 8.2.

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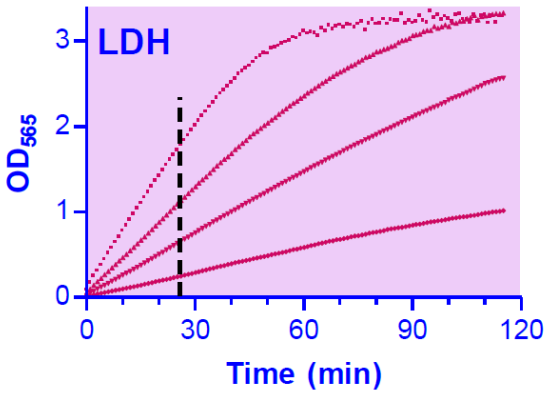
Sample Data

Samples were assayed using the 96-well plate protocol. The LDH activity (IU/L) was 41 for a human serum, 220 for rat serum and 88 for fetal bovine serum, respectively.



Kinetics of LDH reaction in 96-well plate assay with increasing serum concentration

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