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User's Manual and Instructions

Lactate Dehydrogenase Kit (Z5030039)

Colorimetric Kinetic Determination of Lactate Dehydrogenase Activity

DESCRIPTION

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 IU/L, linear up to 200 IU/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.

KIT CONTENTS (100 tests in 96-well plates)

Substrate Buffer: 20 mL, pH 8.2
NAD Solution: 1 mL PMS Solution: 2 mL
MTT Solution: 2 mL Calibrator: 10 mL (equivalent to 200 IU/L)

Storage conditions. Kits are shipped at room temperature. Store all components at -20°C upon receiving. Shelf life of at least 6 months (see expiry dates on labels).

Precautions: reagents are for research use only. Normal precautions for laboratory reagents should be exercised while using the reagents. Please refer to Material Safety Data Sheet for detailed information.

PROCEDURES

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 room temperature. The Color Reagent is prepared by mixing for each 96-well assay, 18 µL MTT Solution, 8 µL NAD Solution and 8 µL PMS Solution. Fresh reconstitution is recommended. The serum or plasma is prepared for each 96 well assay by diluting 3 µL serum with 12 uL H_2O.

Procedure using 96-well plate:

1. Transfer 200 µL H_2O (OD_{H2O}) and 200 µL Calibrator (OD_{CAL}) solution into wells of a clear flat bottom 96-well plate.
2. Transfer 10 µL diluted sample, 30 µL Color Reagent and 160 µL Substrate Buffer into other wells. Tap plate briefly to mix.
3. Read OD_{565nm} (OD_{S0}), and again after 25 min (OD_{S25}) on a plate reader.

Procedure using Cuvette:

1. Transfer 50 µL samples into 1-cm cuvettes.
2. Pipet 150 µL Color Reagent and 800 µL Substrate Buffer to samples. Mix briefly.
3. Read sample OD_{565nm} shortly after the mixing (OD_{S0}), and again after 25 min (OD_{S25}).
4. Read OD_{565nm} for 1 mL water (OD_{H2O}) and Calibrator (OD_{CAL}).

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

CALCULATION

LDH activity of the sample is calculated as

$$\text{LDH Activity} = \frac{OD_{S25} - OD_{S0}}{OD_{CAL} - OD_{H2O}} \times \frac{2}{\text{Sample Vol } (\mu L)} \times 200 \times n \text{ (IU/L)}$$

OD_{S25} and OD_{S0} are OD_{565nm} values of sample at 25 min and 0 min. OD_{CAL} and OD_{H20} are OD_{565nm} values of the Calibrator and water. *n* is the dilution factor. Sample Vol is the volume (µL) of the undiluted sample added per well. Unit definition: 1 Unit (IU) of LDH will catalyze the conversion of 1 µmole of lactate to pyruvate per min at pH 8.2.

MATERIALS REQUIRED, BUT NOT PROVIDED

Pipeting devices and accessories (e.g. multi-channel pipettor).

Procedure using 96-well plate:

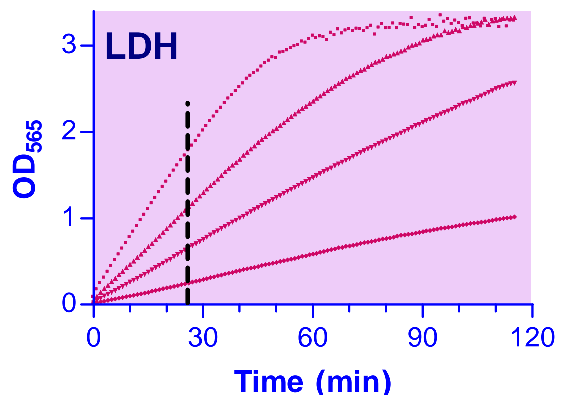
Clear bottom 96-well plates (e.g. Corning Costar) and plate reader.

Procedure using cuvette:

Spectrophotometer and cuvets for measuring OD 565nm.

EXAMPLES

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

LITERATURE

1. Babson, AL and Babson, SR. (1973) Kinetic Colorimetric Measurement of Serum Lactate Dehydrogenase Activity. *Clin Chem*. 19(7):766-9.
2. Karlsen RL, Norgaard L, Guldbrandsen EB (1981). A rapid method for the determination of urea stable lactate dehydrogenase on the 'Cobas Bio' centrifugal analyser.Scand J Clin Lab Invest. 41(5):513-6.
3. Coley HM, Lewandowicz G, Sargent JM, Verrill MW (1997). Chemosensitivity testing of fresh and continuous tumor cell cultures using lactate dehydrogenase.Anticancer Res. 17(1A):231-6.