

QuantiChrom™ Lactate Dehydrogenase Kit (DLDH-100)

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:** 1.5 mL

MTT Solution: 1.5 mL **Calibrator:** 10 mL

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

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 $10,000 \times g$ for 15 min at 4°C . Remove supernatant for assay.

Cell Lysate: collect cells by centrifugation at $2,000 \times g$ 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 $10,000 \times g$ 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 Working Reagent is prepared by mixing for each 96-well assay, 14 μL MTT Solution, 8 μL NAD Solution, 8 μL PMS Solution and 170 μL Substrate Buffer. Fresh reconstitution is recommended.

Procedure using 96-well plate:

1. Transfer 200 μL H_2O ($\text{OD}_{\text{H}_2\text{O}}$) and 200 μL Calibrator (OD_{CAL}) solution into wells of a clear flat bottom 96-well plate.
2. Transfer 10 μL sample, 190 μL Working Reagent into the sample wells. Tap plate briefly to mix.
3. Read $\text{OD}_{565\text{nm}}$ (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 950 μL Working Reagent to samples. Mix briefly.
3. Read sample $\text{OD}_{565\text{nm}}$ shortly after the mixing (OD_{S0}), and again after 25 min (OD_{S25}).
4. Read $\text{OD}_{565\text{nm}}$ for 1 mL water ($\text{OD}_{\text{H}_2\text{O}}$) and Calibrator (OD_{CAL}).

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

CALCULATION

$$\text{LDH Activity} = \frac{\text{OD}_{\text{S25}} - \text{OD}_{\text{S0}}}{\epsilon_{\text{mtt}} \cdot l} \times \frac{\text{Reaction Vol } (\mu\text{L})}{\text{Time} \cdot \text{Sample Vol } (\mu\text{L})} \times n$$

$$= 43.68 \times \frac{\text{OD}_{\text{S25}} - \text{OD}_{\text{S0}}}{\text{OD}_{\text{CAL}} - \text{OD}_{\text{H}_2\text{O}}} \times n \quad (\text{IU/L})$$

OD_{S25} and OD_{S0} are $\text{OD}_{565\text{nm}}$ values of sample at 25 min and 0 min. ϵ_{mtt} is the molar absorption coefficient of reduced MTT. l is the light pathlength which is calculated from the calibrator. OD_{CAL} and $\text{OD}_{\text{H}_2\text{O}}$ are $\text{OD}_{565\text{nm}}$ values of the Calibrator and water. Reaction Vol and Sample Vol are 200 μL and 10 μL , respectively. n is the dilution factor. 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:

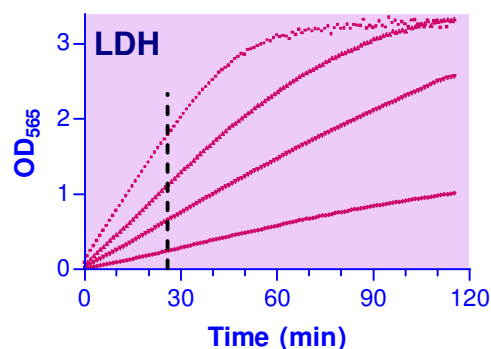
Clear bottom 96-well plates (e.g. Corning Costar) and plate reader.

Procedure using cuvette:

Spectrophotometer and cuvetts for measuring $\text{OD}_{565\text{nm}}$.

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

PUBLICATIONS

1. Stentz R., et al. (2010). Controlled protein release from viable *Lactococcus lactis* cells. *Appl. Environ. Microbiol.* 76(9): 3026-3031.
2. Lu F., et al. (2008). The effect of C1 inhibitor on intestinal ischemia and reperfusion injury. *Am. J. Physiol. Gastrointest. Liver Physiol.* 295(5): G1042-G1049.
3. Mittal R., et al. (2009). Brain damage in newborn rat model of meningitis by *Enterobacter sakazakii*: a role for outer membrane protein A. *Lab. Invest.* 89(3): 263-277.