Technical Bulletin

Lactate Dehydrogenase Assay Kit

Catalogue Number MAK464

Product 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.

The linear detection range of the kit is 2-200 U/L. The kit is suitable for lactate dehydrogenase activity determination in serum, plasma, and other sources. It is also suitable for the characterization and quality control of LDH production, as well as for the screening and evaluation of LDH modulators in drug discovery.

Components

The kit is sufficient for 100 colorimetric assays in 96-well plates.

- Substrate Buffer — 20 mL
  Catalogue Number MAK464A
- Diaphorase — 120 µL
  Catalogue Number MAK464B
- NAD Solution — 1 mL
  Catalogue Number MAK464C
- MTT Solution — 1.5 mL
  Catalogue Number MAK464D
- Calibrator — 1.5 mL
  Catalogue Number MAK464E

Reagents and Equipment Required but Not Provided

- Pipetting devices and accessories (such as, multichannel pipettor)
- Spectrophotometric multiwell plate reader
- Clear flat-bottom 96-well plates. Cell culture or tissue culture treated plates are **not** recommended
- Dounce tissue grinder set
  (Catalogue Number D9063 or equivalent)
- Refrigerated microcentrifuge capable of RCF ≥ 10,000 × *g*
- Phosphate Buffered Saline (PBS)
  (Catalogue Number P3813 or equivalent)
- EDTA disodium salt
  (Catalogue Number ED2SS or equivalent)

Precautions and Disclaimer

For Research Use Only. Not for use in diagnostic procedures. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

The kit is shipped on wet ice. Store components at -20 °C.

Preparation Instructions

Briefly centrifuge small vials prior to opening. Assay can be executed at room temperature or 37 °C. Equilibrate all components to desired temperature prior to use.

Procedure

All samples and standards should be run in duplicate.

Sample Preparation

Serum and plasma are assayed directly.

Tissue

1. Prior to dissection, rinse tissue in phosphate buffered saline, pH 7.4, to remove blood.
2. Homogenize tissue in 5 mL of buffer containing 100 mM potassium phosphate, pH 7.0, and 2 mM EDTA, per gram of tissue.
3. Centrifuge at 10,000 × *g* for 15 minutes at 4 °C.
4. Remove supernatant and retain for assay.

Cell Lysate

1. Collect cells by centrifugation at 2,000 × *g* for 5 minutes at 4 °C.
2. For adherent cells, **do not** harvest cells using proteolytic enzymes. Instead, use a rubber policeman or cell scraper.
3. Homogenize or sonicate cells in an appropriate volume of cold buffer containing 100 mM potassium phosphate, pH 7.0, and 2 mM EDTA.
4. Centrifuge at 10,000 × *g* for 15 minutes at 4 °C.
5. Remove supernatant and retain for assay.

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

Working Reagent

For each well, prepare 198 µL of Working Reagent according to Table 1.

Table 1.
Preparation of Working Reagent

Reagent	Volume
MTT Solution	14 µL
NAD Solution	8 µL
Diaphorase	1 µL
Substrate Buffer	175 µL

Assay Reaction

Note: This assay is based on a kinetic reaction. To ensure identical incubation time, addition of Working Reagent to wells should be quick and mixing should be brief but thorough. Use of a multi-channel pipettor is recommended.

1. Set the plate reader for the desired assay temperature (room temperature [25 °C] or 37 °C).
2. Transfer 200 µL of purified water (Blank) and 200 µL of Calibrator solution into separate wells of a clear flat bottom 96-well plate.
3. Transfer 10 µL of each Sample into separate wells of the plate.
4. Add 190 µL of Working Reagent to all wells. Tap plate lightly to mix.

Measurement

Immediately read the optical density (OD) of each well at 565 nm at zero and 25 minutes (OD_{0Min} and OD_{25Min} respectively). Alternatively, using the plate reader's kinetic mode, monitor the OD for 25 minutes.

Results

Lactic Dehydrogenase (LDH) activity is calculated as follows:

LDH Activity (U/L) =

$$\frac{OD_{S25} - OD_{S0}}{\varepsilon_{MTT}\ x\ L} \times \frac{Reaction\ Vol\ (\mu L)}{\text{Time x Sample Vol } (\mu L)} \times DF$$

$$= 43.68 \times \frac{OD_{S25} - OD_{S0}}{OD_{CAL} - OD_{H2O}}\ x\ DF$$

where

OD_{S25} = OD value at 565 nm of Sample at 25 minutes

OD_{S0} = OD value at 565 nm of Sample at 0 minutes

ε_{MTT} = Molar absorption coefficient of reduced MTT

L = Light pathlength which is calculated from the Calibrator

OD_{CAL} and OD_{H20} = OD values at 565 nm of the Calibrator and Blank (water)

Reaction Vol = 200 µL

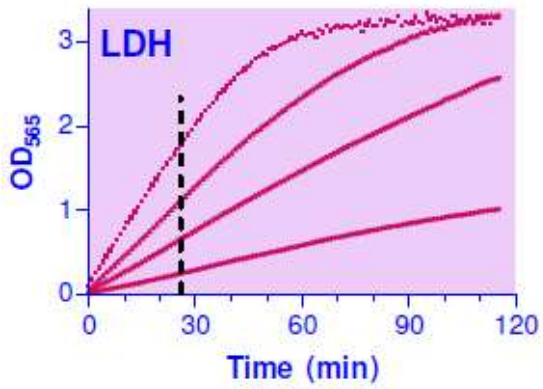
Sample Vol = 10 µL

DF = Dilution Factor of the Sample

Note: If the Sample LDH activity exceeds 200 U/L, dilute Samples in water and repeat the assay.

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

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



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