Tribioscience

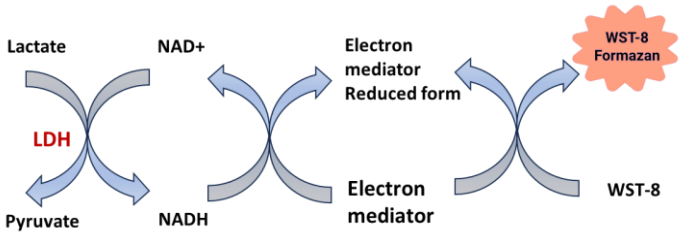
LDH Lactate Dehydrogenase Colorimetric Assay (Catalog: TBS2012, 500 Assays, Store at -20°C)

DESCRIPTION

Lactate dehydrogenase (LDH) is a cytosolic enzyme present in nearly all living cells. It is an oxidoreductase catalyzing the interconversion of lactate and pyruvate. LDH is released upon damage of the cytoplasmic membrane. The measurement of LDH release is a well-accepted assay to estimate tissue or cell damage and cell cytotoxicity.

The Tribo™ LDH Colorimetric Assay Kit uses WST-8, which is oxidized to generate formazan to measure the activity of LDH released from damaged cells. The generated signal at OD 460 nm is proportional to the LDH released from the lysed cells. The kit provides the easiest and most accurate approach to measure LDH activity.

Fig. 1: LDH Assay Principle



APPLICATIONS

1) Quantify lactate dehydrogenase in a variety of samples such as serum, plasma tissues, cells, and cell culture medium.
2) This kit is designed in a format for a variety of sample types. To measure LDH released into a cell culture medium in a cytotoxicity experiment, we recommend TBS2002, LDH Cytotoxicity Colorimetric Assay.

KIT CONTENTS FOR 500 TESTS:

Name	Size (500 tests)
LDH Substrate Mix	6 mL
LDH Assay Buffer	20 mL
LDH Dye Probe	5 mL
LDH standard stock (1250 Units/mL)	50 µL

Storage conditions: Store the Reagent at –20°C protected from light. Shelf life: 12 months.

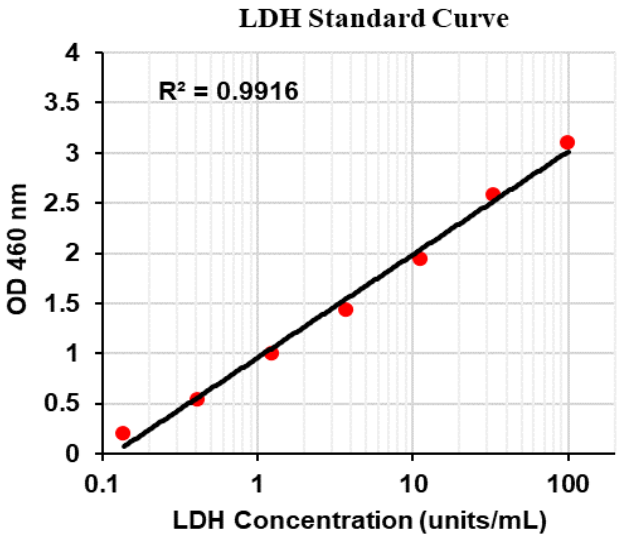
PROCEDURES

1. Equilibrate all the kit components until room temperature before starting the experiment.
2. Prepare the LDH working solution: add 17 mL of LDH Assay Buffer, 6 mL of Substrate Mix to, mix well and then add 4 mL LDH Dye Probe to make the LDH working solution in a 50 mL conical tube, mix all the reagents completely. This is LDH working solution (Note: the volume of the LDH working solution can be changed proportionally with each component part to meet your need, such as 1/5 volume of working solution for 1 plate). Store unused portion of Substrate Mix and the Dye at -20°C.
3. Prepare LDH standard: add 20 µL LDH stock to 230 µL of LDH assay buffer as tube #1, then make a 3-fold serial dilution by adding 100 µL of the LDH to 200 µL of LDH assay buffer, label as tube #2 to #7, #8 is assay buffer only without LDH. The LDH standards from tube #1 to #7 are 100, 33.3, 11.1, 3.7, 1.2, 0.4, and 0.14 units/mL. Add 50 µL of the LDH standards to the wells (duplicate) of a 96-well microplate.
4. Add 50 µL of LDH working solution to each well of samples and the LDH standards. Gently mix the reagents by shaking.
5. Incubate the microplate at 37°C for 1 hour with shaking and protected from light.
6. Measure the OD intensity at 460 nm.
7. Calculate the LDH concentration by the typical LDH standard curve as follows: Y=Ax + B

   Y is the $OD_{460\ nm}$ reading and X is the concentration of LDH (Units/mL).

   The concentration of LDH (Units/mL) = $e^{(OD460\ nm\ -\ B)/A}$

Fig. 2: LDH Standard Curve



RELATIVE PRODUCTS

Resazurin Cell Viability Kit (TBS2001)
CCK-8 Cell Viability Assay (TBS2022)
ATP Colorimetric/Fluorometric Assay Kit (TBS2010)
ADP/ATP Ratio Assay Kit (Bioluminescent (TBS2015)
ADP Colorimetric/Fluorometric Assay Kit (TBS2020)
Caspase-3 Colorimetric Assay kit (TBS2030)
Caspase-3 Fluorometric Assay kit (TBS2035)

This product is for research only.

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