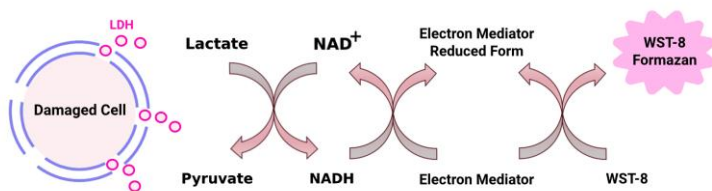


DESCRIPTION

Lactate dehydrogenase (LDH) is a stable cytoplasmic enzyme which is present in most cells. It is released upon damage to the cytoplasmic membrane. The measurement of LDH release is a well-accepted assay to estimate cell membrane integrity and quantify cell cytotoxicity.

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

Fig. 1: LDH Assay Principle



APPLICATIONS

- 1) Cell Proliferation.
- 2) Cytotoxicity and LDH activity in cells and tissue.

KIT CONTENTS FOR 500 TESTS:

Name	Size (500 tests)
LDH Substrate Mix	6 mL
LDH Assay Buffer	20 mL
LDH Dye Probe Mix	5 mL
Cell Lysis Buffer	6.0 mL
LDH Standard stock (1250 Units/mL)	50 µL

Storage conditions: Store the Reagent at -20°C protected from light. Shelf life: 6 months.

PROCEDURES

1. Seed 1X10²⁻⁴ cells/well in 96-well microplate in 100 µL/well with or without test compounds. Set up the treatment in duplicate manner. The blank control is non-cell control with media and compound. All experiments must have parallel wells for lysis assay.
2. Equilibrate all the kit components until room temperature before starting the experiment.

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.

4. Add 10 µL PBS/well to half of the parallel wells of samples and controls. Add 10 µL/well of lysis buffer to the rest half of the parallel wells of samples and controls. Incubate the plate on a microplate shaker for 10-20 min at 50-100 rpm to facilitate cell lysis.
5. Prepare LDH standard (**optional**): add 20 µL LDH Standard Stock to 230 µL of cell culture medium with cell lysis buffer (medium: lysis buffer =10 :1) (tube #1), then make a 3-fold serial dilution by adding 100 µL of the LDH Standard to 200 µL of culture medium with lysis buffer, label as tube #2 to #7, #8 is medium 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, respectively.
6. Add 50 µL of the LDH standards to the indicated wells (duplicate) of a microplate.
7. Transfer 50 µL of supernatant from each well of cell culture plate (after cell lysis or PBS only) to a new microplate.
8. Add 50 µL of LDH working solution to each well of cell supernatants and the LDH standards. Gently mix the reagents by shaking.
9. Incubate the microplate at 37°C for 1 hour with shaking and protected from light.
10. Measure the OD intensity at 460 nm.
11. Calculate the percentage of cytotoxicity for tested compounds according to the following formula:

$$\% \text{ Cytotoxicity} = 100 \times (\text{OD}_{\text{unlyzed cell}}) / (\text{OD}_{\text{lyzed cells}})$$

OD_{unlyzed cell} = OD_{460 nm} reading of unlysed cells - background reading (Blank Control);

OD_{lyzed cell} = OD_{460 nm} reading of lysed cells - background reading (Blank Control).

12. Calculate the LDH concentration by the typical LDH standard curve as follows: Y=AX^B

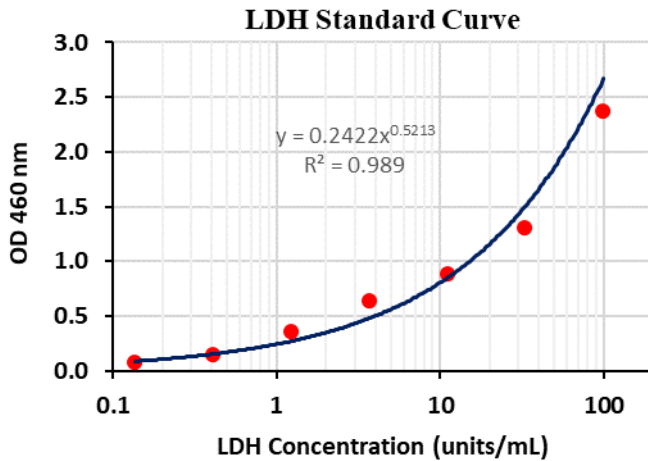
Y is the OD_{460 nm} reading and X is the concentration of LDH (Units/mL).

$$\text{The concentration of LDH (Units/mL)} = \sqrt[B]{Y/A}$$

LDH Cytotoxicity Colorimetric Assay (Catalog: TBS2002, 500 Assays, Store at -20 °C)

3. Prepare the LDH working solution: add 17 mL of LDH Assay Buffer, 6 mL of Substrate Mix, and 4mL Dye Probe into 50 mL Conical Tube, mix well. This mixture

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 use only.