Rev. 4

LDH Cytotoxicity Assay (LDH)

Catalog #8078
500 Tests

Product Description

Lactate dehydrogenase (LDH) is a soluble cytosolic enzyme present in most eukaryotic cells. LDH releases into culture medium upon cell death due to damage of plasma membrane. The increase of the LDH activity in culture supernatant is proportional to the number of lysed cells. LDH Cytotoxicity Assay kit provides a colorimetric method to measure LDH activity using a reaction cocktail containing lactate, NAD^+, Diaphorase and INT. LDH catalyzes the reduction of NAD^+ to NADH in the presence of L-lactate, while the formation of NADH can be measured in a coupled reaction in which the tetrazolium salt INT is reduced to a red formazan product. The amount of the highly colored and soluble formazan can be measured at 490 nm spectrophotometrically.

Kit Components

Cat. #	# of vials	Component	Quantity	Storage
8078a	1	LDH Positive Control	20 µL	4°C
8078b	1	Sodium Lactate, 10×	0.5 mL	4°C
8078c	1	INT, 10×	0.5 mL	-20°C
8078d	1	Substrate Mix	5 mL	-20°C
8078e	1	Stop Solution	10 mL	4°C
8078f	1	Reconstitution Solution	0.2 mL	4°C
8078g	1	10X Lysis Buffer	1 mL	4°C

Materials supplied by user:

Colorimetric microplate reader
96-well plate

Quality Control

The LDH Cytotoxicity Assay was applied to primary cells seeded at different densities, with and without Triton X-100, demonstrating a linear relationship between OD490nm and cell number (Figure 1).

Product Use

LDH cytotoxicity assay (LDH) is designed for the rapid and sensitive detection of LDH released from damaged cells. This kit is for research use only and is not approved for human or animal use, or for application in clinical or *in vitro* diagnostic procedures.

Shipping

Dry ice.

Storage
Upon receipt, store the Sodium Lactate (Cat. #8078b) Stop Solution (Cat. #8078e), Cell Lysis Buffer (Cat. # 8078g) and Reconstitution Solution (Cat. #8078f) at 4°C. Store Lyophilized LDH standard (Cat. #8078a), INT (Cat. #8078c), and Substrate Mix (Cat. #8078d) at -20°C.

Procedure (96-well plate)
Note: Serum, like fetal calf serum and fetal bovine serum, added to the growth medium contains LDH, leading to unintended background color changes (OD_{490nm}) unrelated to cell death. To minimize background interference caused by serum LDH, consider using the minimum serum percentage appropriate for each cell type without compromising cell viability. Additionally, you have the option of subtracting the signal from medium-only controls to further correct for any remaining background signal.

A. Performing cytotoxicity assay

1. Plate the optimal number of cells/well in 100 µL of medium in triplicate wells in a 96-well tissue culture plate. **Note**: Cell density may need to be optimized.
   In addition to the test samples, three maximum LDH activity cultures and three spontaneous LDH activity cultures in medium should be included.
2. Incubate cells overnight at 37°C in the cell culture incubator.
3. After overnight incubation, prepare samples according to the following:
   a. Add 10 µL of sterile water to the spontaneous LDH activity of triplicate wells containing cells, then mix by gently tapping.
   b. Add 10 µL of vehicle containing chemical compound to one set of triplicate wells of cells (test samples).
   c. To the one set of triplicate wells of maximum LDH activity do not add any components.
   d. Add 100 µL medium/well into triplicate wells to determine LDH background activity present in serum used for media supplementation. The background value has to be subtracted from all other values.
4. Incubate the experimental plate in a cell culture incubator for desired experimental time.
5. Add 10 µL of 10X Lysis buffer to the maximum LDH activity, then mix by gently tapping. Note: Do not create bubbles when pipetting because bubbles prevent accurate readings.
6. Incubate the plate in a cell culture incubator for 10 minutes.
7. *(Optional but recommended)* Centrifuge the 96-well culture plate at 400 x g for 5 minutes.
8. Transfer 50 µl of supernatant from each sample medium (test, maximum LDH activity and spontaneous LDH activity) to a 96-well flat bottom plate in triplicate wells.

B. Preparation of LDH positive control

1. Take 1 µl of LDH positive control (Cat. #8078a) and mix it with 4 µl of PBS. Add 2 µl of diluted stock to each positive control well with 48 µl of culture medium.

C. Measurements

1. To make 50 µl of working reaction mixture for each well of 96-well plate, add 1 µl of sodium lactate, 1 µl of INT and 10 µl of substrate mix to 38 µl of PBS.
2. Add 50 µl of working reaction mixture into each well of the 96-well test plate containing LDH positive control, test samples, or maximum and spontaneous LDH activity wells. Incubate for 20 minutes at room temperature in the dark.
   Note: The reaction time can be decreased or increased depending on the color development. The plate can be read at multiple time points until the desired reading is

observed. The maximum LDH activity should be $OD_{450nm} < 2.0$, while the spontaneous LDH activity should be $OD_{450nm} < 0.1$.

3. Stop the reaction with 20 µl of Stop Solution per well of 96-well plate.
4. Read the absorbance at 490 nm with an ELISA plate reader.

D. Calculations

1. Average the OD_{490} nm of replicate wells of each test sample, control, and blank. Subtract the average OD_{490} nm value of the blank from the average OD_{490} nm values obtained with all other samples.
2. The relative cytotoxicity of test compounds can be calculated based on the OD_{490nm} values as follows:

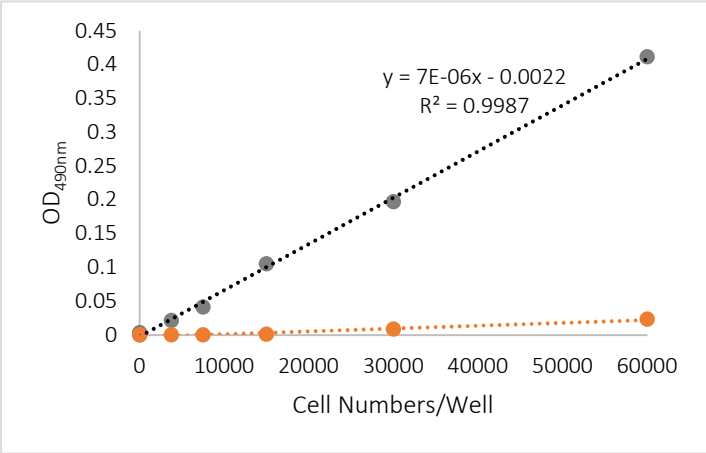

***Figure 1**. LDH assay is applied to Primary cells cultured at different densities and treated with (gray line) and without Triton X-100 (orange line).*

$$Cytotoxicity\ (\%) = \frac{OD_{490\text{nm, test sample}} - OD_{490\text{nm, spontaneous LDH activity}}}{OD_{490\text{nm, maximum LDH activity}} - OD_{490\text{nm, spontaneous LDH activity}}} * 10$$