LDH Cytotoxicity Assay

Cat. No. 8078
500 Tests

Introduction

Lactate dehydrogenase (LDH), which is a soluble cytosolic enzyme present in most eukaryotic cells, 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. ScienCell™ LDH Cytotoxicity Assay kit provides a colorimetric method to measure LDH activity using a reaction cocktails containing lactate, NAD^+, diaphrose and INT. LDH catalyses 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. No.	# of vials	Reagent	Quantity	Storage
8078a	1	LDH standard	1 ml	4°C
8078b	1	Sodium Lactate, 10×	1 ml	4°C
8078c	1	INT, 10×	1 ml	-20°C
8078d	1	Substrate Mix	10 ml	-20°C
8078e	1	Sodium Oxymate	10 ml	4°C

Quality Control

Data from ScienCell™ LDH Cytotoxicity Assay of LDH solutions with concentrations ranging from 500 to 7.8 mU/ml shows a linear relationship between OD_{490nm} and LDH concentration (Figure 1). ScienCell™ LDH Cytotoxicity Assay is also applied to Human Astrocytes (HAs) seeded at different densities with (positive control) and without (negative control) Triton X-100 (Figure 2).

Procedures (96-well plate)

A. Cell culture setup

1. Seed cells in a 96-well culture plate in 200 µl of culture medium with or without test compounds. Culture the cells in a CO_2 humidified incubator at 37°C for the desired period of time. We recommend that you prepare at least 3 replicates for each test sample. Besides test samples, three positive control cultures in medium with 1% Triton X-100 and three negative control cultures in medium without any test compounds or Triton X-100 should be included.

B. Preparation of LDH standard (optional)

1. Add 2 µl of LDH stock to 498 µl of PBS to make a 500 µl solution of 1000 mU/ml LDH.
2. Obtain 8 test tubes, add 450 µl of culture medium into each tube and label them #1 through #8.
3. Add 450 µl of the 1000 mU/ml LDH solution into tube #1 and mix well to get the 500 mU/ml LDH standard.
4. Transfer 450 µl of the 500 mU/ml LDH standard from tube #1 to tube #2 and mix well to get the 250 mU/ml LDH standard.

5. Repeat step 4 for tubes #3-7 to serially dilute the LDH standards. Do not add any LDH to tube #8, which serves as the blank.
6. Obtain a 96-well test plate, prepare 3 replicates (A, B, C) of each LDH standard by aliquoting 150 µl/well of each LDH standard into triplicate wells of the 96-well test plate, according to the following plate format:

	#1	#2	#3	#4	#5	#6	#7	#8
A	500 mU/ml	250 mU/ml	125 mU/ml	62.5 mU/ml	31.8 mU/ml	15.6 mU/ml	7.8 mU/ml	Blank
B	500 mU/ml	250 mU/ml	125 mU/ml	62.5 mU/ml	31.8.mU/ml	15.6 mU/ml	7.8 mU/ml	Blank
C	500 mU/ml	250 mU/ml	125 mU/ml	62.5 mU/ml	31.8 mU/ml	15.6 mU/ml	7.8 mU/ml	Blank

C. Preparation of test samples and +/-controls

1. Centrifuge the 96-well culture plate at 400 g for 5 min and transfer 150 µl of supernatant from each well (test, positive and negative control wells) to the corresponding well of the 96-well test plate.

D. Measurements

1. To make 60 µl of working reaction mixture for each well of 96-well plate, add 2 µl of sodium lactate, 2 µl of INT and 20 µl of substrate mix to 36 µl of PBS.
2. Add 60 µl of working reaction mixture into each well of the 96-well test plate containing LDH standard, test samples, or controls. Incubate for 20 minutes at room temperature in dark.
3. Stop the reaction with 20 µl of sodium oxymate solution per well of 96-well plate.
4. Read the absorbance at 490 nm with an ELISA plate reader.

E. Calculations

1. Average the OD_{490nm} of replicate wells of each LDH standard, test sample, control, and blank. Subtract the average OD_{490nm} value of the blank from the average OD_{490nm} values obtained with all other samples.
2. Based on the calibrated OD_{490nm} of the LDH standard, make a standard curve by plotting OD_{490nm} as a function of LDH concentration. (See Figure 1 for a typical standard curve.) Determine the equation and R^2 value of the trend line.
3. Suppose the equation of the trend line of the standard curve is $y = Ax + B$, calculate the LDH concentration of test samples and controls as follows:

$$[LDH] = \frac{OD_{490nm} - B}{A}$$

4. Calculate the cytotoxicity of the test compounds as follows:

$$Cytotoxicity \quad (\%) = \frac{[LDH]_{test\ \ sample} - [LDH]_{negative\ \ control}}{[LDH]_{positive\ \ control} - [LDH]_{negative\ \ control}} \times 100$$

5. If the exact LDH concentration is not needed, the measurement of the LDH standard curve can be skipped and the relative cytotoxicity of test compounds can also be calculated based on the OD_{490nm} values as follows:

$$Cytotoxicity \quad (\%) = \frac{OD_{490nm,\ test\ sample} - OD_{490nm,\ negative\ control}}{OD_{490nm,\ positive\ control} - OD_{490nm,\ negative\ control}} \times 100$$

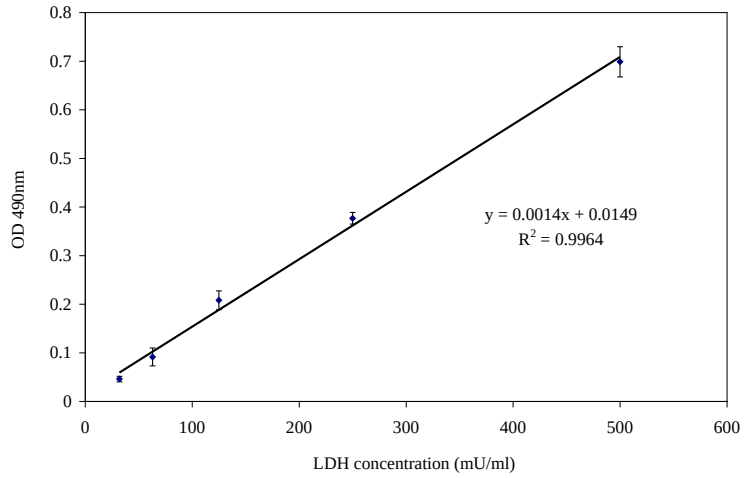

Figure 1. A typical LDH standard curve measured by ScienCell™ LDH Cytotoxicity Assay.

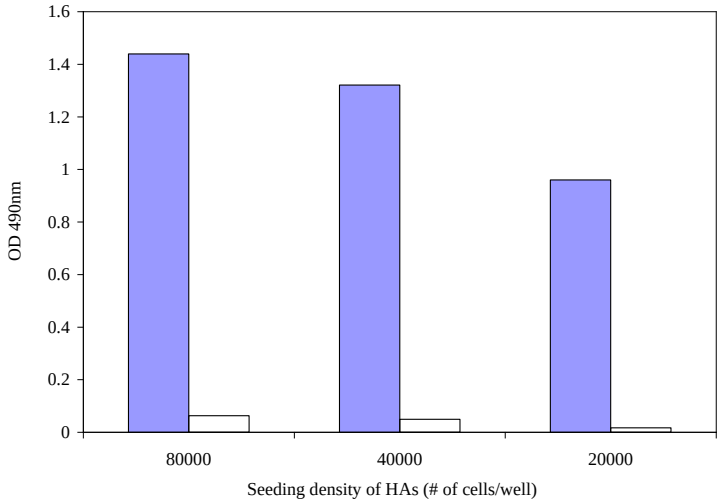

Figure 2. ScienCell™ LDH assay is applied to Human Astrocytes (HAs) cultured with (solid bar, positive control) and without (open bar, negative control) Triton X-100.