Rev. 0

LDH Cytotoxicity Assay-Fluorescence (LDH-FL)

Catalog #8778
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 LDH activity in culture supernatant is proportional to the number of lysed cells. The LDH Cytotoxicity Assay Kit, Fluorescence (LDH-FL) is designed to measure lactate dehydrogenase (LDH) activity through a fluorescence-based reaction. This assay utilizes a reaction cocktail containing lactate, NAD^+, diaphorase, and resazurin. LDH catalyzes the conversion of NAD^+ to NADH in the presence of L-lactate. Subsequently, diaphorase uses the NADH produced to reduce resazurin to resorufin, which can be detected via fluorescence with an excitation wavelength of 560 nm and an emission wavelength of 590 nm. The amount of resorufin formed is directly proportional to the LDH released into the culture medium, providing a measure of cytotoxicity. This kit offers higher sensitivity in a shorter timeframe and can detect lower concentrations of LDH activity compared to the colorimetric version.

Kit Components

Cat. #	# of vials	Component	Quantity	Storage
8778a	1	LDH positive control	1 vial	4°C
8778b	1	Sodium Lactate, 10X	0.5 mL	4°C
8778c	1	Resazurin, 10X	0.5 mL	-20°C
8778d	1	Substrate Mix	5 mL	-20°C
8778e	1	Stop Solution	10 mL	4°C
8778f	1	Lysis Buffer, 10x	1 mL	4°C

Materials supplied by user:

Fluorescent microplate reader (excitation 530-560/emission 580-600)
96-well plate

Quality Control

Data from LDH Cytotoxicity Assay of LDH positive control with concentrations ranging from 1.9 to 125 mU/mL shows a linear relationship between fluorescent intensity and LDH concentration (Figure 1). This assay is also applied to primary cells seeded at different densities with (Maximum LDH release) and without (Spontaneous LDH release) Lysis Buffer (Figure 2).

Product Use

LDH cytotoxicity assay kit (LDH) is designed for the rapid and sensitive detection of LDH to assess cytotoxicity induced by chemical compounds and to evaluate cell-mediated cytotoxicity. The kit is suitable for use with various mammalian cell types, including 3D cell models, and in various sample types, including serum, plasma, and cell lysates. 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 LDH positive control (Cat. #8778a), Sodium Lactate (Cat. #8778b) Stop Solution (Cat. #8778e), Cell Lysis Buffer (Cat. # 8778f) at 4°C. Store Resazurin (Cat. #8778c), and Substrate Mix (Cat. #8778d) at -20°C.

Procedure (96-well plate)
Note: Serum, like fetal calf serum and fetal bovine serum, added to the growth medium contains LDH, which increases the background and decrease the sensitivity of the assay. 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 release cultures and three spontaneous LDH release cultures 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 release 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 release 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 (cat #8778f) to the maximum LDH release, 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.
7. *(Optional but recommended)* Centrifuge the 96-well culture plate at 400 x g for 5 minutes and transfer 50 µl of supernatant from each sample medium (test, Maximum and spontaneous LDH release) to a 96-well flat bottom plate in triplicate wells.

B. Preparation of LDH standard (optional)

1. Add 1 µl of LDH positive control (cat #8778a) to 19 µl of PBS (can be stored for 3 days at 4°C).
2. Take 1 µl of the diluted LDH to make a 200 µl solution of 250 mU/mL LDH.
3. Obtain 7 test tubes, add 200 µl of culture medium into each tube and label them #1-7.
4. Add 200 µl of the 250 mU/mL LDH solution into tube #1 and mix well to get the 125 mU/mL LDH standard.

5. Transfer 200 µl of the 250 mU/mL LDH standard from tube #1 to tube #2 and mix well to get the 62.5 mU/mL LDH standard.
6. Repeat step 4 for tubes #3-6 to serially dilute the LDH standards. Do not add any LDH to tube #7, which serves as the blank.
7. Obtain a 96-well test plate, aliquot 50 µl/well of each LDH standard into triplicate wells of the 96-well test plate.

	1	**2**	**3**	**4**	**5**	**6**	**7**
LDH mU/mL	125	62.5	31.8	15.6	7.8	3.9	Blank

C. Measurements

1. To make 50 µl of 2X working reaction mixture for each well of 96-well plate, add 1 µl of sodium lactate (cat #8778b) 1 µl of Resazurin (cat #8778c) and 10 µl of substrate mix (cat #8778d) to 38 µl of PBS.
   Note: *The sensitivity of the assay can be increased by reducing the Resazurin concentration in the 2X working reaction mixture. For example, add 0.5 µL of Resazurin per well. Note that this change decreases the maximum attainable signal.*
2. Add 50 µl of working reaction mixture into each well of the 96-well test plate containing LDH standard, test samples and controls.
3. Measure the fluorescence (excitation 530-560, emission 580-600) at 1 minute interval for 15-20 minutes) to determine the optimal time point for the particular experiment.
4. Alternatively, incubate the plate in the dark for 10 minutes, then stop the reaction by adding 20 µl of Stop Solution (cat #8778e) per well of the 96-well plate if you prefer to read the plate later (within 1-2 hours).

D. Calculations

1. Average the fluorescence intensity of replicate wells of each LDH standard, test sample, control, and blank. Subtract the average fluorescent signal value of the blank from the average values obtained with all other samples.
2. Based on the calibrated fluorescent intensity of the LDH standard, make a standard curve by plotting fluorescent intensity 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. Assume 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.

$$[LDH] = \frac{Fluorescent\ intensity - B}{A}$$

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

$$Cytotoxicity\ (\%) = \frac{[LDH]_{\text{test sample}} - [LDH]_{\text{spontaneous LDH release}}}{[LDH]_{\text{maximum LDH release}} - [LDH]_{\text{spontaneous LDH release}}} * 100$$

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 fluorescent intensities as follows:

$$Cytotoxicity\ (\%) = \frac{\text{Test Sample} - \text{Spontaneous LDH release}}{\text{Maximum LDH release} - \text{Spontaneous LDH release}} * 100$$

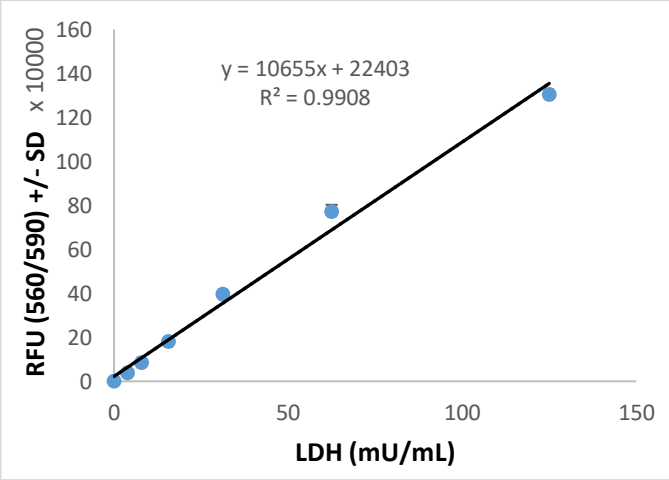

Figure 1. LDH standard curve measured by LDH-FL Assay.

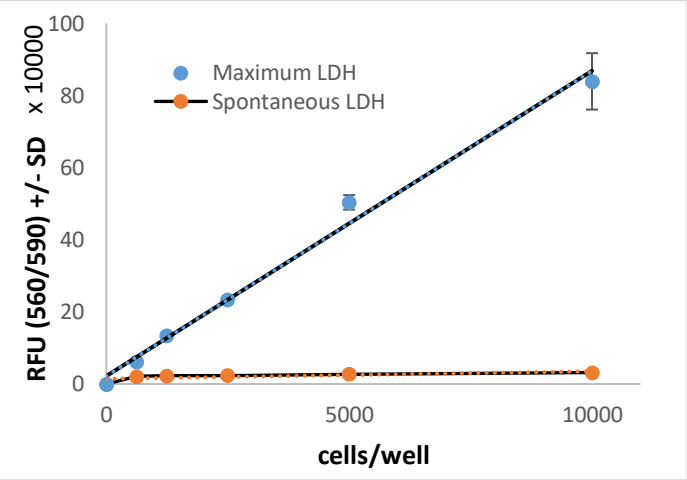

Figure 2. LDH-FL assay is applied to human primary cells cultured with and without cell lysis buffer.