DHL™ Cell Cytotoxicity Assay Kit

Fluorimetric

Catalog #	71302
Unit Size	1 kit
Kit Size	500 assays (96-well) or 1000 assays (384-well)

This kit provides a convenient fluorescent method to detect lactate dehydrogenase (LDH) released from damaged cells. Released cytoplasmic LDH is a well-established indicator of cell membrane damage and cell cytotoxicity. The fluorescence signal can be monitored at excitation/emission=530-560 nm/590 nm. The kit has the following features:

- ***Convenient Format:*** All essential assay components are included.
- ***Optimized Performance:*** Optimal conditions for the detection of cell cytotoxicity.
- ***Enhanced Value:*** Less expensive than the sum of individual components.
- ***High Speed:*** Minimal hands-on time.
- ***Assured Reliability:*** Detailed protocol and references are provided

USA and Canada Ordering Information

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Technical Support
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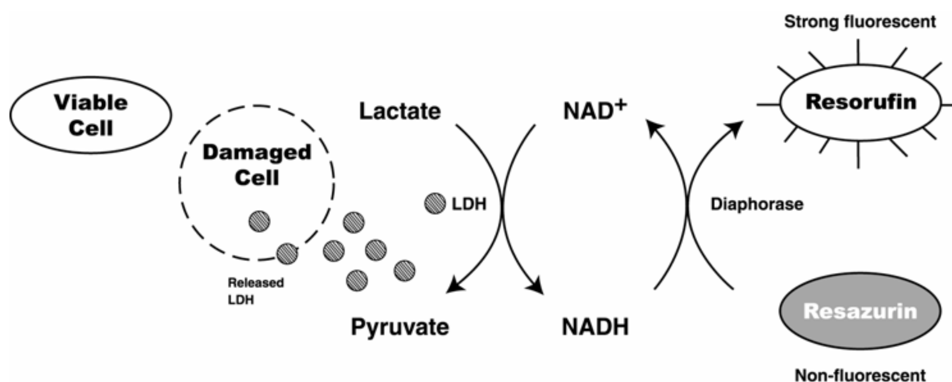
International Ordering Information

A list of international distributors is available at www.anaspec.com.

INTRODUCTION

The damage of cell membrane leads to release of cytoplasmic enzymes. The measurement of lactate dehydrogenase (LDH) release is a well-accepted assay to estimate cell membrane integrity and quantify cell cytotoxicity[1-5]. LDH release assay has been proven to correlate very well with traditional ^{51}Cr release assay and trypan blue staining[2;6].

The EnzoLyte™ Cell Cytotoxicity Assay Kit uses resazurin as a fluorogenic indicator to measure the activity of LDH released from damaged cells (Scheme 1). The fluorescent signal is proportional to the number of dead cells (r^2>0.95). Meanwhile, the viable cells produce negligible fluorescent signal under same condition (Figure 1). Therefore, the assay can be performed in a mixture of damaged and viable cells. The kit is suitable for high throughput screening of cytotoxicity of a variety of compounds.



Scheme 1. In a cell population that co-exists viable cells and damaged cells, the DHL™ cell cytotoxicity assay kit only detects the dehydrogenases (e.g. LDH) activity released from damaged cell, not those in live cells. By using an enzyme-coupled reaction, the dehydrogenases in the medium will convert non-fluorescent resazurin to strongly fluorescent resorufin, which can be detected at emission of 590 nm with the excitation at 530-560nm.

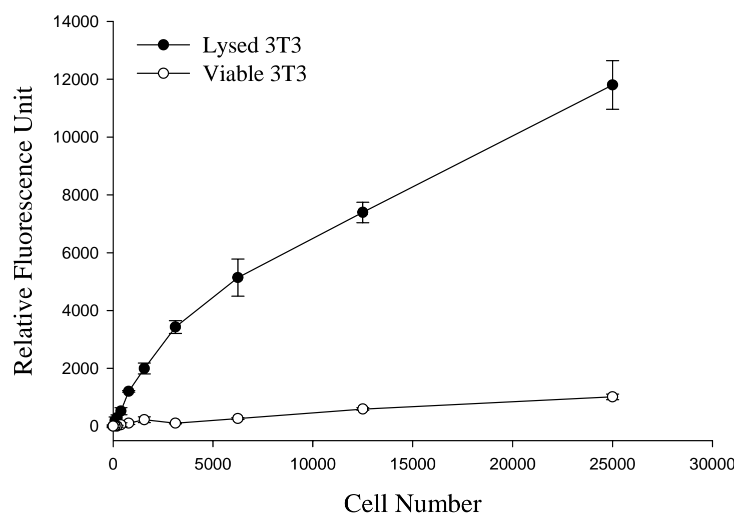


Figure 1. The increment of fluorescence signal is correlated with the increment of lysed cells. 3T3 cells were seeded into 96-well plate. 10 µL of lysis solution was added to the cells to release cytoplasmic LDH (lysed 3T3), while 10 µL of growth medium was added to another sets of wells (viable 3T3). 50 µL of LDH assay solution was added. Tthe fluorescence signal was monitored at Ex/Em=530±30/590±30 nm 10 minutes later. The assay can detect as little as 97 lysed 3T3 cells (>±3S.D.), while the living cells produce little fluorescence signal.

KIT COMPONENTS, STORAGE AND HANDLING

Note: Store all the kit components at -20℃, and keep component A and B from light.

Component A: Assay mixture (1 bottle)

Component B: Assay buffer (30 mL)

Component C: Lysis solution (10 mL)

Component D: Stop solution (20 mL)

OTHER MATERIALS REQUIRED (BUT NOT PROVIDED)

96 or 384-well microplate: Tissue culture microplate with black wall and clear bottom.
Fluorescence microplate reader: Capable of detecting emission at 590 nm with excitation at 530-560 nm.

STANDARD OPERATION PROTOCOL

Note: Please use protocol A or B based on your needs.

Protocol A. Assay the cytotoxicity effect of test compounds.

1. Prepare cell culture.

- Seed $1X10^{2-4}$ cells per well in a microplate. Add test compounds and then culture cells in a 37°C incubator for the desired exposure period. The total volume suggested for a 96-well plate is 100 µL. The total volume suggested for a 384-well plate is 50 µL. All samples must have at least four parallel wells.
- Set up the following controls at the same time. All controls must have at least four parallel wells.

➢ Positive control contains cells and known proliferation or cytotoxicity inducer.

➢ Negative control contains cells but no test compounds.

➢ Vehicle control contains cells and the vehicle used to deliver test compounds.

➢ Non-cell control contains growth medium but no cells.

Note: LDH contained in serum will contribute to background fluorescence.

➢ Test compound control contains growth medium and test compound. Some test compounds have strong autofluorescence and may give false results.

Note: Match the total volume of all the controls to 100 µL for a 96-well plate or 50 µL for a 384-well plate by growth medium.

2. Prepare LDH assay solutions.

Note: Warm up all the kit components until thawed at 22-25℃ before starting the experiments. Warm up the stop solution (component D) at 37℃ to dissolve all the precipitation.

- LDH assay solution: add 25 mL of assay buffer (component B) to one vial of assay mixture (component A), which is sufficient for five 96-well plate. Mix the reagents completely. Store unused portion of LDH assay solution at -20°C.

3. Assay LDH activity.

- Retrieve the cells from 37°C incubator and incubate at 22-25°C for 20-30 minutes.

Note: It is important to equilibrate the temperature of the cells to 22-25℃ before the assay.

- For the 96-well plate, add 10 µL/well of lysis solution (component C) to half of the parallel wells of samples and controls. Meanwhile, add 10 µL/well of Hank's balance solution (HBSS) or phosphate-buffered saline (PBS) to the rest half of the parallel wells of samples and controls. For

the 384-well plate, add 5 μL/well of lysis solution (component C) to half of the parallel wells of samples and controls. Meanwhile, add 5 μL/well of Hank's balance solution (HBSS) or phosphate-buffered saline (PBS) to the rest half of the parallel wells of samples and controls.

- Incubate the plate on a microplate shaker for 1-2 min at 50-100 rpm to facilitate cell lysing.
- For 96-well plate, add 50 μL of LDH assay solution to each well. Mix the reagents by shaking gently for 30 seconds. (Add 25 μL of LDH assay solution for a 384-well plate).
- Incubate the reaction at 22-25°C for 10 minutes.
- (Optional) Add 20 μL of stop solution (component D) to each well (5 μL for 384-well plate).
- Measure fluorescence intensity at Ex/Em=530-560 nm/590 nm immediately.

4. Perform data analysis:

- The fluorescence reading from the non-cell control well is the background fluorescence. The readings from other wells need to be subtracted with this background fluorescence.
- Calculate the percentage of cytotoxicity for samples and controls according to the following formula:

$$\% \text{ Cytotoxicity} = 100 \text{ X } (LDH_{cytotoxic})/(LDH_{maximal})$$

The fluorescence reading from unlysed cells represents $LDH_{cytotoxic}$.
The fluorescence reading from lysed cells represents $LDH_{maximal}$.

Note: Negative control wells still have $LDH_{cytotoxic}$ reading caused by spontaneous release of LDH[2] *or cells autolysis.*

<u>Protocol B.</u> Cytotoxicity Assay for Co-culture System.

1. Prepare cells according to Table 1.

- Seed effector cell and target cells at desired testing concentration and ratio in a microplate. Match the total volume to 100 µL per well for a 96-well plate (50 µL for a 384-well plate).
- Set up the following controls at the same time.
  - <u>Target cell control</u> contains target cells at the concentration used in test wells. Prepare at least four parallel wells
  - <u>Effector cell control</u> contains effector cells at the concentration used in test wells.
  - <u>Non-cell control</u> contains growth medium but no cells.

  <u>Note:</u> LDH contained in serum will contribute to background fluorescence.

  <u>Note</u>: Match the total volume of all the controls to 100 µL for a 96-well plate or 50 µL for a 384-well plate by growth medium.
- Culture cells in a 37°C incubator for the desired exposure period.

2. Prepare LDH assay solutions.

<u>Note</u>: Warm up all the kit components until thawed at 22-25 °C before starting the experiments.

- <u>LDH assay solution</u>: add 25 mL of assay buffer (component B) to one vial of assay mixture (component A), which is sufficient for five 96-well plate. Mix the reagents completely. Store unused portion of LDH assay solution at -20°C.

3. Initiate enzymatic reaction.

- Retrieve the cells from 37°C incubator and incubate at 22-25°C for 20-30 minute.

  <u>Note</u>: It is important to equilibrate the temperature of the cells to 22-25 °C before the assay.
- For the 96-well plate, add 10 µL/well of lysis solution (component C) to half of the parallel wells of the target cell control. For the 384-well plate, add 5 µL/well of lysis solution (component C) to half of the parallel wells of the target cell control.
- Incubate the plate on a microplate shaker for 1-2 min at 50-100 rpm to facilitate cell lysing.
- For the 96-well plate, add 50 µL of LDH assay solution to each well. Mix the reagents by shaking gently for 30 seconds. (Add 25 µL of LDH assay solution for the 384-well plate).
- Incubate the reaction at 22-25°C for 10 minutes.
- (Optional) Add 20 µL of stop solution (component D) to each well (5 µL for 384-well plate). Mix the reagent by shaking 10 second.
- Measure fluorescence intensity at Ex/Em=530-560 nm/590 nm immediately.
- Continue to data analysis.

4. Perform data analysis:

- The fluorescence reading from the non-cell control well is the background fluorescence. The readings from other wells need to be subtracted with this background fluorescence.
- Calculate percent cytotoxicity according to the following formula[7]:

$$\%\ \text{Cytotoxicity} = 100 \times \frac{(\text{LDH}_{\text{cytotoxic}} - \text{Spontaneous LDH}_{\text{target cell}} - \text{Spontaneous LDH}_{\text{effector cell}})}{(\text{Maximal LDH}_{\text{target cell}} - \text{Spontaneous LDH}_{\text{target cell}})}$$

The fluorescence reading from testing wells containing both target and effector cell represents $\text{LDH}_{\text{cytotoxic}}$.
The fluorescence reading from lysed target cell control represents Maximal $\text{LDH}_{\text{target cell}}$.
The fluorescence reading from unlysed target cell control represents Spontaneous $\text{LDH}_{\text{target cell}}$.
The fluorescence reading from unlysed effector cell control represents Spontaneous $\text{LDH}_{\text{effector cell}}$.

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