SensoLyte® Cell Cytotoxicity Assay Kit *Fluorimetric*

Revision number: 1.2	*Last updated: October2014*
Catalog #	**AS-71303**
Kit Size	5,000 Assays (96-well plate)

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

Kit Components, Storage and Handling

Component	Description	Quantity
Component A	Assay mixture	10 bottles
Component B	Assay buffer	25 mL X 10 bottles
Component C	Lysis solution	100 mL
Component D	Stop solution	100 mL

Other Materials Required (but not provided)

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

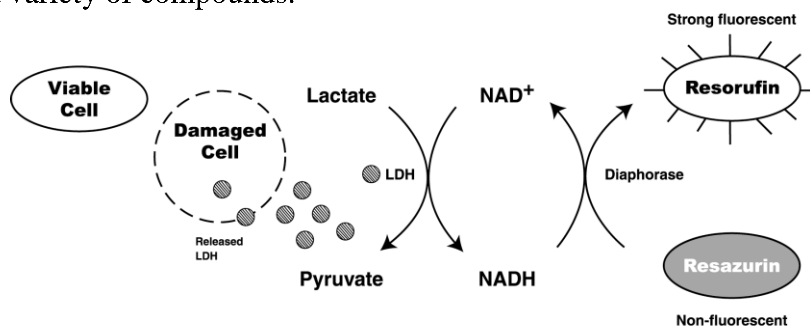
Storage and Handling

- Store all kit components at -20°C.
- For convenience, Components C and D can be stored at room temperature.

Introduction

Cell membrane damage leads to the release of cytoplasmic enzymes, and 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 the traditional ^{51}Cr release assay and trypan blue staining.[2,6]

The SensoLyte® Cell Cytotoxicity Assay Kit uses resazurin as a fluorogenic indicator for measuring 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). In contrast, viable cells produce negligible fluorescent signal under same condition (Figure 1). Therefore, this 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 of mixed viable cells and damaged cells, the SensoLyte® cell cytotoxicity assay kit only detects the dehydrogenases (e.g. LDH) activity released from damaged cell, and not those in live cells. In the enzyme-coupled reaction, dehydrogenases in the medium convert non-fluorescent resazurin to the strongly fluorescent resorufin, which can be monitored at Ex/Em= 530-560 nm/590 nm.

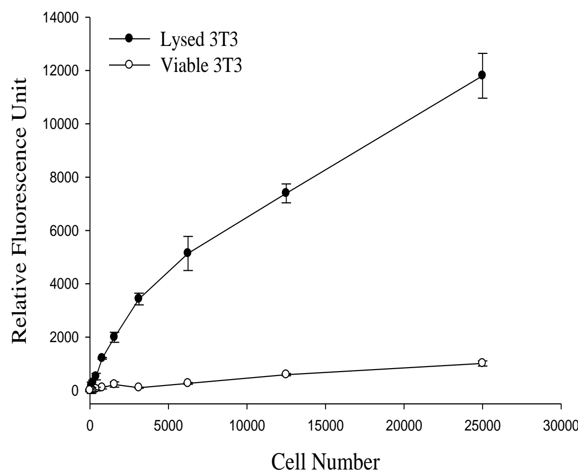


Figure 1. An increase of fluorescence signal is correlated with an increase of lysed cells.
3T3 cells were seeded into a 96-well plate. 10 µL of lysis solution was added to the cells to release cytoplasmic LDH (lysed 3T3), and 10 µL of growth medium was added to another sets of wells (viable 3T3). 50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

Protocol

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

Protocol A. Cytotoxicity assay for test compounds

1. Prepare cell culture and test compound treatment.

1.1 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 length of time. The total volume suggested is 100 µL/well. All samples must have at least four parallel wells.

1.2 Simultaneously set up the following controls as deemed necessary. 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.

1.3 Bring the total volume of all the controls to 100 µL per well using growth medium.

2. Prepare LDH assay solution.

Note: Warm all the kit components until thawed at 22-25°C before starting the experiments. Warm the stop solution (Component D) at 37°C to dissolve all precipitates.

2.1 LDH assay solution: Add 25 mL of assay buffer (Component B) to one vial of assay mixture (Component A) – this is sufficient for five 96-well plates. Mix the reagents completely. Store unused portion of LDH assay solution at -20°C.

3. Measure released LDH activity.

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

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

3.2 Add 10 µL per well of lysis solution (Component C) to half of the parallel wells of samples and controls. Add 10 µL per well of Hank's balance solution (HBSS) or phosphate-buffered saline (PBS) to the rest half of the parallel wells of samples and controls.

3.3 Incubate the plate on a microplate shaker for 1-2 min at 50-100 rpm to facilitate cell lyses.

3.4 Add 50 µL of LDH assay solution to each well. Mix the reagents by shaking gently for 30 seconds.

3.5 Incubate the reaction at 22-25°C for 10 min.

3.6 Optional: Add 20 µL per well of stop solution (Component D) immediately to each well. Mix the reagent by shaking 30 sec. If the stop solution looks cloudy, warm it up in a 37°C water bath to dissolve the precipitates before using it.

Note: The stop solution will slightly increase the fluorescence signal. The signal will be stable for 24 h after the addition of stop solution.

3.7 Measure fluorescence intensity at Ex/Em=530-560 nm/590 nm.

4. Perform data analysis.

4.1 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.

4.2 Calculate the percentage of cytotoxicity for samples and controls according to the following formula:

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

Where:

$LDH_{cytotoxic}$ = fluorescence reading from unlyzed cells.
$LDH_{maximal}$ = fluorescence reading from lyzed cells.

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

Protocol B. Cytotoxicity Assay for Co-culture System

1. Prepare co-cultured cells.

1.1 Seed effectors cell and target cells at the desired testing concentration and ratio in a microplate. Bring the total volume to 100 µL/well.

1.2 Simultaneously set up the following controls as deemed necessary.

- Target cell control contains target cells at the concentration used in test wells. Prepare at least four parallel wells
- Effectors cell control contains effectors cells at the concentration used in test wells.
- Non-cell control contains growth medium but no cells.

Note 1: LDH contained in serum will contribute to background fluorescence.
Note 2: Bring the total volume of all the controls to 100 µL using growth medium.

1.3 Culture cells in a 37°C incubator for the desired time period.

2. Prepare LDH assay solution.

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

2.1 LDH assay solution: Add 25 mL of assay buffer (Component B) to one vial of assay mixture (Component A) - this is sufficient for five 96-well plates. Mix the reagents completely. Store unused portion of LDH assay solution at -20°C.

3. Measure released LDH activity.

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

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

3.2 Add 10 µL per well of lysis solution (Component C) to half of the parallel wells of the target cell control.

3.3 Incubate the plate on a microplate shaker for 1-2 min at 50-100 rpm to facilitate cell lysis.

3.4 Add 50 µL of LDH assay solution to each well. Mix the reagents by shaking gently for 30 sec.

3.5 Incubate the reaction at 22-25°C for 10 min.

3.6 Optional: Add 20 µL per well of stop solution (Component D). Mix the reagent by shaking 30 sec.

Note: The stop solution will slightly increase the fluorescence signal. The signal will be stable for 24 h after the addition of stop solution.

3.7 Measure fluorescence intensity at Ex/Em=530-560 nm/590 nm.

4. Perform data analysis.

4.1 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.

4.2 Calculate percent cytotoxicity according to the following formula:[7]

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

Where:
$LDH_{cytotoxic}$ = fluorescence reading from testing wells containing both target and effectors cell.
Maximal $LDH_{target\ cell}$ = fluorescence reading from lyzed target cell control.
Spontaneous $LDH_{target\ cell}$ = fluorescence reading from unlyzed target cell control.
Spontaneous $LDH_{effector\ cell}$ = fluorescence reading from unlyzed effector cell control.

References:

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7. Konjevic, G. et al. *J Immunol Methods* 200, 199 (1997).