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Cytotoxicity LDH Assay Kit

1 Contents

Components	HY-K1090-100T	HY-K1090-500T
Working Solution	5.5 mL	27.5 mL
Stop Solution	5.5 mL	27.5 mL
Lysis Solution	1.1 mL	5.5 mL

2 Introduction

The loss of cell membrane integrity due to cell death or membrane damage results in release of certain soluble, cytosolic enzymes. Therefore, acute cytotoxicity can be measured by measuring activity of these enzymes released into the culture supernatant. Lactate dehydragenase (LDH) is a stable enzyme, present in all cell types, and rapidly released into the cell medium upon damage of the plasma membrane. LDH, therefore, is the most widely used marker in cytotoxicity study.

LDH oxidizes lactate to generate NADH, which then reacts with certain dye to generate yellow color. The intensity of the generated color correlates directly with the cell number lysed, which is indicative of cytotoxicity. LDH activity can be easily quantified by spectrophotometer or plate reader at 490 nm.

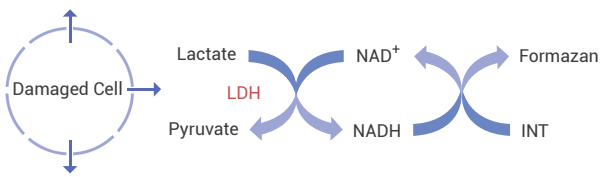

Fig 1. Schematic of MCE Cytotoxicity LDH Assay Kit.

MCE Cytotoxicity LDH Assay Kit can be directly added to cells to detect LDH activity in the culture supernatant (one-step, direct method). Or you can separate the culture supernatant from cell and use this kit to detect LDH activity (indirect method). The separated cells can be used for other experiments.

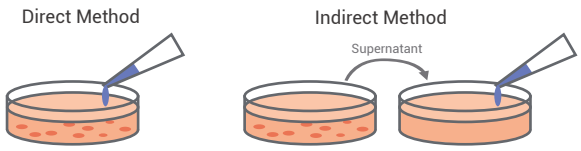

Fig 2. Detect LDH activity by direct and indirect method.

3 General Protocol

1. Preparation of controls

1.1 High Control: Measure the maximum releasable LDH in the cells by the addition of Lysis Solution.

1.2 High Blank Control: Measure the LDH activity in the assay medium and Lysis Solution.

1.3 Low Control: Measure the spontaneous LDH release from untreated normal cells.

1.4 Background Blank: Measure the LDH activity in the assay medium. The absorbance value obtained in this control is subtracted from other values.

Table 1: Preparation of controls

	Samples	High Control	High Blank Control	Low Control	Background Blank
Medium	-	-	100 µL	10 µL	110 µL
Cell Suspension	100 µL	100 µL	-	100 µL	-
Drug	10 µL	-	-	-	-
Lysis Solution	-	10 µL	10 µL	-	-

2. Determine the optimum cell number for LDH cytotoxicity assay

2.1 Inoculate cell suspension (100 µL/well) in a 96-well plate. Pre-incubate the plate in a humidified incubator (e.g., at 37°C, 5% CO_2).

2.2 One set of the serial dilutions is lysed and used to determine the Maximum LDH Release. Add 10 µL of Lysis Solution to the Maximum LDH Release dilution series, then mix by gentle tapping.

2.3 The second set of serial dilutions is used to determine the Spontaneous LDH release. Add 10 μL of medium to the Spontaneous LDH Release dilution series of triplicate wells containing cells, then mix by gentle tapping.

2.4 Incubate the plate for 30 minutes in the incubator.

2.5 **Direct Method:** Aspirate 50 μL of culture supernatant from each well, and use the remaining culture supernatant as the detection object. Add 50 μL of Working solution to each sample well, then mix by gentle tapping.

Indirect Method: Transfer 50 μL of each suspension to a new 96-well plate. Transfer 50 μL of Working solution to each sample well, then mix by gentle tapping.

Note: When you need cells for other experiments, please choose indirect method. Otherwise, direct method is simple and efficient.

2.6 Incubate the plate at room temperature for 30 minutes protected from light.

2.7 Add 50 μL of Stop Solution to each sample well, and then mix by gentle tapping. Measure the absorbance at 490 nm using a microplate reader.

3. Cell Proliferation and Cytotoxicity Assay

3.1 Seed cells in a 96-well plate at a density of 10^4 - 10^5 cells/well in 100 μL of medium with or without compounds to be tested. Culture the cells in a CO_2 incubator at 37°C for 24 hours.

3.2 Add 10 μL of various concentrations of substances to be tested to the plate.

3.3 Incubate the plate for an appropriate length of time (e.g., 6, 12, 24 or 48 hours) in the incubator.

3.4 One set of the serial dilutions is lysed and used to determine the Maximum LDH Release. Add 10 μL of Lysis Solution to the Maximum LDH Release dilution series, then mix by gentle tapping.

3.5 The second set of serial dilutions is used to determine the Spontaneous LDH release. Add 10 μL of medium to the Spontaneous LDH Release dilution series of triplicate wells containing cells, then mix by gentle tapping.

3.6 Incubate the plate for 30 minutes in the incubator.

3.7 **Direct method:** Aspirate 50 μL of culture supernatant from each well, and use the remaining culture supernatant as the detection object. Add 50 μL of Working solution to each sample well, then mix by gentle tapping.

Indirect method: Transfer 50 μL of each suspension to a new 96-well plate. Transfer 50 μL of Working solution to each sample well, then mix by gentle tapping.

Note: When you need cells for other experiments, please choose indirect method. Otherwise, direct method is simple and efficient.

3.8 Incubate the plate at room temperature for 30 minutes protected from light.

3.9 Add 50 μL of Stop Solution to each sample well, and then mix by gentle tapping. Measure the absorbance at 490 nm using a microplate reader.

Note: Detect viable cell number by CCK8 Kit (HY-K0301) if you need.

4. Calculation of cytotoxicity

Determine the percent of cytotoxicity by the following equation.

Cytotoxicity (%) = [(X-Z)/(Y-Z)] × 100%

X: Absorbance of Samples - Background Blank

Y: Absorbance of High Control - High Blank Control

Z: Absorbance of Low Control - Background Blank

4 Storage

Stored at -20°C protecting from light, and is stable for up to 12 months.

5 Precautions

1. Be careful not to introduce bubbles to the wells, since they interfere with the O.D. reading.
2. It is recommended to conduct the assay in the presence of low serum (e.g., 1%) or 1% bovine serum albumin (BSA) (w/v). Both human and animal sera contain various amounts of LDH, which may increase background absorbance in the assay.
3. The amount of LDH is dependent on the cell types. We recommend carrying out a preliminary experiment to optimize the cell concentration.
4. This product is for R&D use only, not for drug, household, or other uses.
5. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.