Product Manual

LDH Cytotoxicity WST Assay

Catalog #: ENZ-KIT157

ASSAY FOR THE DETERMINATION OF CYTOTOXICITY BY LACTATE DEHYDROGENASE ACTIVITY

500 Tests

5 x 500 Tests

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Product Manual

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Please read entire booklet before proceeding with the assay.

Carefully note the handling and storage conditions of each kit component.

Please contact Enzo Life Sciences Technical Support if necessary.

INTRODUCTION

LDH Cytotoxicity WST Assay is a kit for the determination of cytotoxicity by measuring lactate dehydrogenase (LDH) activity released from damaged cells. LDH is a stable cytoplasmic enzyme presented in all types of cells and released into the cell culture medium through damaged plasma membrane. LDH Cytotoxicity WST Assay can be used to measure the released LDH according to the following scheme. LDH catalyzes dehydrogenation of lactate to pyruvate thereby reducing NAD to NADH. NADH reduces a water-soluble tetrazolium salt (WST) in the presence of an electron mediator to produce an orange formazan dye. The amount of the formazan dye thus formed is proportional to that of released LDH into the medium, which is an indication of cytotoxicity.

Since LDH Cytotoxicity WST Assay neither reflects the activity of living cells nor is harmful to cells, cytotoxicity can be measured with the living cells (homogeneous assay). In addition, non-homogeneous assay that is performed by using the cell culture supernatant is also possible.

Unlike competitive products, the reconstituted Working Solution is stable under refrigerated condition and the Working Solution can be used for long periods as ready-to-use solution after the preparation.

PRINCIPLE OF THE ASSAY

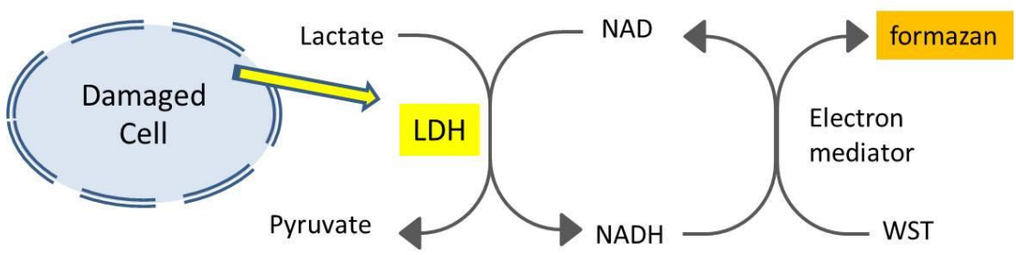

Fig.1 Principle of cytotoxicity measurement

MATERIALS SUPPLIED

	500 tests	5 x 500 tests
Dye Mixture	Bottle × 1	Bottle × 5
Assay Buffer	55 mL × 1	55 mL × 5
Lysis Buffer	5.5 mL × 1	5.5 mL × 5
Stop Solution	27.5 mL × 1	27.5 mL × 5

ADDITIONAL MATERIALS AND EQUIPMENT NEEDED

- CO_2 incubator
- Microplate reader (490 nm filter)
- 96-well tissue culture plate (flat-bottomed). For suspension cells in non-homogeneous assay: Round or V-bottomed plate
- 20 and 100-200 µL multichannel pipettes
- 96-well optically clear plate (flat-bottomed) for non-homogeneous assay

PRECAUTIONS


Handle with care

- This kit contains a glass bottle with an aluminum cap. Use protective gloves and be cautious in handling.
- The amount of LDH is dependent on cell type. We recommend carrying out a preliminary experiment to optimize the cell concentration which is determined by maximum absorbance difference at 490 nm between low and high controls.

REAGENTS AND SAMPLE PREPARATION

1. Add 5 mL of Assay Buffer to the Dye Mixture vial. Close the cap and dissolve the contents completely.
2. Add the whole volume of the mixture prepared in Step 1 to Assay Buffer bottle.
3. Store the Working Solution at 0°C - 5°C and protect it from light. It is stable for 2 months.


Storage temp

ASSAY PROTOCOL – HOMOGENEOUS ASSAY

Optimization of Cell Concentration

1. Collect cells and wash them with the assay medium. Prepare cell suspension to 5.0×10^5 cells/mL in the assay medium.
2. Add 100 µL of the assay medium to each well of a flat-bottom 96-well tissue culture plate.
3. Prepare 2-fold serial dilution of each well in triplicate set of wells for the high-control, low-control and background control (medium only) (Refer to Fig.2 for the plate arrangement).

Serial Dilution Procedure:

a. Add 100 µL of the cell suspension (5.0×10^5 cells/mL) to the first well [A] and mix by pipetting. This well contains the maximum number of cells (2.5×10^4 cells/well).

b. Transfer 100 µL from the first well to the next well [B] and mix by pipetting.

c. Repeat this procedure and discard 100 µL from the last dilution.

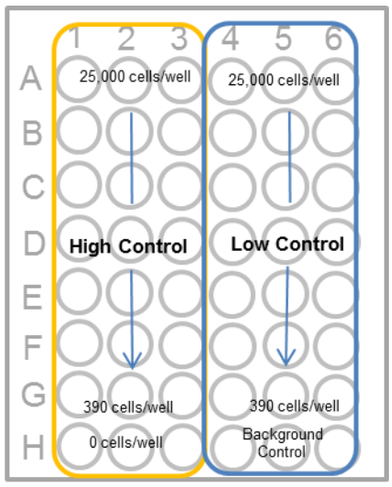

Fig.2 Plate arrangement

4. Incubate the plate at 37°C for an appropriate time in a CO_2 incubator (use the same incubation time in the cytotoxicity assay).
5. Add 10 µL of the Lysis Buffer to each well of the high control.
6. Incubate the plate at 37°C for 30 minutes in a CO_2 incubator.


Protect from light

7. Add 100 µL of the Working Solution to each well. Protect the plate from light and incubate it at room temperature for 30 minutes.
8. Add 50 µL of the Stop Solution to each well.
9. Measure the absorbance at 490 nm by a microplate reader.

Cytotoxicity Assay

1. Add 50 µL of cell suspension to each well of a flat-bottom 96-well tissue culture plate.

   For adherent cells: Incubate the plate at 37°C overnight in a CO_2 incubator to allow the cells to adhere tightly. Remove the medium and add 50 µL of fresh assay medium.

2. Add 50 µL of assay medium containing test substance that adjusted to the desired concentration (Refer to Table 1).

Table 1: Amount of each solution (Homogeneous Assay)

	Test Substance	High Control	Low Control	Background Control
Assay medium	-	50 µL	50 µL	100 µL
Cell suspension	50 µL	50 µL	50 µL	-
Test substance in culture medium	50 µL	-	-	-
Lysis Buffer	-	10 µL	-	-

** The difference of total volume of test substance and high control does not affect the result.*

3. Incubate the plate at 37°C for an appropriate time period in a CO_2 incubator.
4. Add 10 µL of the Lysis Buffer to each well of the high control. Incubate the plate at 37°C for 30 minutes in a CO_2 incubator.


Protect from light

5. Add 100 µL of the Working Solution to each well. Protect the plate from light and incubate it at room temperature for 30 minutes.
6. Add 50 µL of the Stop Solution to each well.
7. Measure the absorbance at 490 nm using a microplate reader.

Calculation of Cytotoxicity

Calculate the average absorbance from each triplicate set of wells and subtract the background control value from each absorbance value. Calculate the percent cytotoxicity by the following equation:

$$\text{Cytotoxicity}(\%) = \frac{(A-C)}{(B-C)} \times 100$$

A: Test substance
B: High control
C: Low control

ASSAY PROTOCOL – NON-HOMOGENEOUS ASSAY

Optimization of Cell Concentration

1. Collect cells and wash them with the assay medium. Prepare cell suspension to 5.0×10^5 cells/mL in the assay medium.
2. Add 100 µL of the assay medium to each well of a 96-well tissue culture plate. Use round or v-bottomed plate for suspension cells, flat-bottomed plate for adherent cells.
3. Prepare 2-fold serial dilution of each well in triplicate set of wells for the high-control, low-control and background control (medium only) (Refer to Fig. 2 for the plate arrangement).

   Serial Dilution Procedure:

   a. Add 100 µL of the cell suspension (5.0×10^5 cells/mL) to the first well [A] and mix by pipetting. This well contains the maximum number of cells (2.5×10^4 cells/well).

   b. Transfer 100 µL from the first well to the next well [B] and mix by pipetting.

c. Repeat this procedure and discard 100 µL from the last dilution.

4. Add 100 µL of the assay medium to each well.
5. Incubate the plate at 37°C for an appropriate time in a CO_2 incubator (use the same incubation time in the cytotoxicity assay).
6. Add 20 µL of the Lysis Buffer to each well of the high control.
7. Incubate the plate at 37°C for 30 minutes in a CO_2 incubator.
8. Centrifuge the plate at 250×g for 2 minutes to precipitate the cells (for suspension cells).
9. Transfer 100 µL of the supernatant from each well to an optically clear 96-well plate.


Protect from light

10. Add 100 µL of the Working Solution to each well. Protect the plate from light and incubate it at room temperature for 30 minutes.
11. Add 50 µL of the Stop Solution to each well.
12. Measure the absorbance at 490 nm by a microplate reader.

Cytotoxicity Assay

1. Add 100 µL of cell suspension to each well of a flat-bottom 96-well tissue culture plate.

   For adherent cells: Incubate the plate at 37°C overnight in a CO_2 incubator to allow the cells to adhere tightly. Remove the medium and add 100µl of fresh assay medium.

2. Add 100 µL of the assay medium containing test substance that adjusted to the desired concentration (Refer to Table 2).

Table 2: Amount of each solution (Non-Homogeneous Assay)

	Test Substance	High Control	Low Control	Background Control
Assay medium	20 µL	100 µL	120 µL	220 µL
Cell suspension	100 µL	100 µL	100 µL	-
Test substance in culture medium	100 µL	-	-	-
Lysis Buffer	-	20 µL	-	-

3. Incubate the plate at 37°C for an appropriate time period in a CO_2 incubator.
4. Add 20 µL of the Lysis Buffer to each well of the high control. Incubate the plate at 37°C for 30 minutes in a CO_2 incubator.

5. Centrifuge the plate at 250×g for 2 minutes to precipitate the cells (for suspension cells).
6. Transfer 100 µL of the supernatant from each well to each well of a new optically clear 96-well plate.
7. Add 100 µL of the Working Solution to each well. Protect the plate from light and incubate it at room temperature for 30 minutes.
8. Add 50 µL of the Stop Solution to each well.
9. Measure the absorbance at 490 nm by a microplate reader.

Protect from light

10.

Cytotoxicity of Mitomycin C Using HeLa Cells

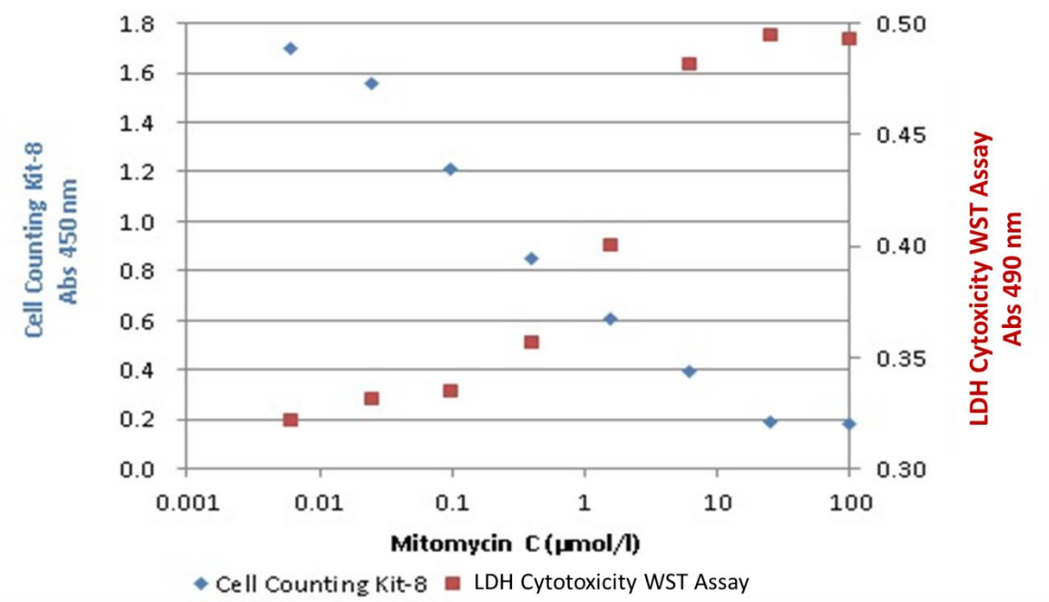

Fig. 3 Cytotoxicity of Mitomycin C Using HeLa cells. Test substance: Mitomycin C, Cell line: HeLa, Culture medium: MEM, 10% FBS, Incubation: 37°C, 5% CO_2, 48 hours

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