Cytotoxicity LDH Assay Kit-WST

interchim Technical Manual

Technical Manual (Japanese version) is available at http://www.dojindo.co.jp/manual/ck12.pdf

General Information

Cytotoxicity LDH Assay Kit-WST is a kit for determination of cytotoxicity by measuring a 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. Cytotoxicity LDH Assay Kit-WST 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 Cytotoxicity LDH Assay Kit-WST 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.

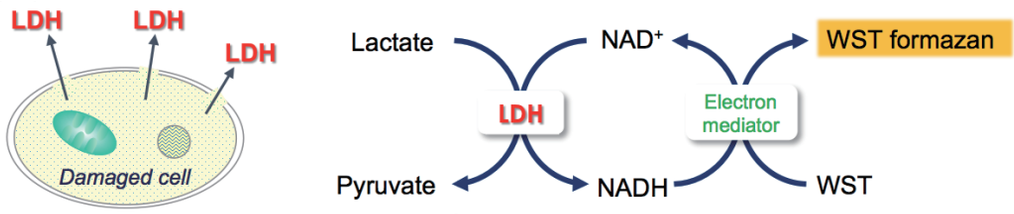
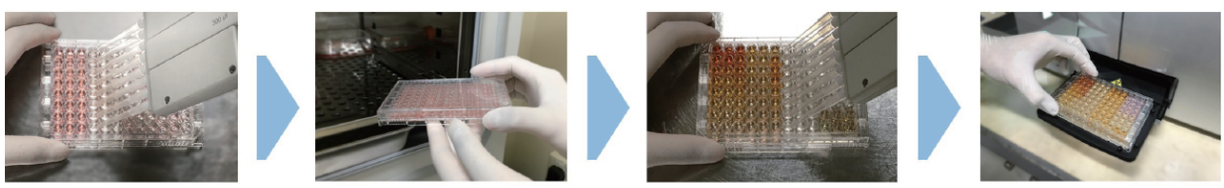


Fig. 1 Principle of cytotoxicity measurement

Procedure



Add Working Solution
(Only for high control wells, add Lysis Buffer prior to the addion of Working solution)

Colorimetric reaction(30 minutes)

Add Stop Solution and stop the colorimetric reaction

Measure the absorbance at 490 nm

Kit Contents

	100 tests	500 tests	2000 tests
Dye Mixture	× 1	× 1	× 4
Assay Buffer	11 ml × 1	55 ml × 1	55 ml × 4
Lysis Buffer	1.1 ml × 1	5.5 ml × 1	5.5 ml × 4
Stop Solution	5.5 ml × 1	27.5 ml × 1	27.5 ml × 4

Storage Condition

Store at 0-5 °C

Required Equipment and Materials

- 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, 100-200 µl multichannel pipettes
- 96-well optically clear plate (flat-bottomed)
  *For non-homogeneous assay

Precaution

- 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 the cell types. We recommend carrying out a preliminary experiment to optimize the cell concentration.

Preparation of Reagent

Working Solution

1) Add appropriate volume of Assay Buffer to the Dye Mixture vial. Close the cap and dissolve the contents completely.
   Add 1 ml and 5 ml of Assay Buffer to the Dye Mixture vial of the unit of 100 tests and 500 tests, respectively.
2) Add the whole volume of the mixture prepared in 1) to Assay Buffer bottle.
   Store the Working Solution at 0-5 °C and protected it from light. It is stable for 6 months.

General Protocol

Homogeneous assay

Optimization of cell concentration

1) Collect cells and wash them with the medium. Prepare cell suspension to 5×10^5 cells/ml in the medium.
2) Add 100 µl of the 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]
   Add the cell suspension (5×10^5 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). Transfer 100 µl from the first well to the next well [B], and mix by pipetting. Repeat this procedure.
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.
7) Add 100 µl of the Working Solution to each well. Protect the plate from light and incubate it at the 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.

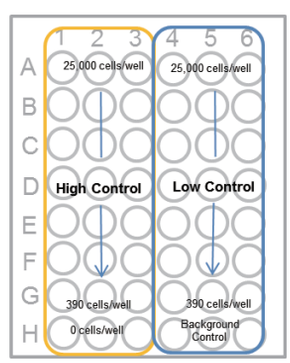


Fig. 2 Plate arrangement

*Plotting the data by setting absorbance for the x-axis and cell concentration for the y-axis to determine the optimum cell concentraiton as reffering below recomendation.
- The difference in the absorbance between high control and low control is at least 0.2.
- The absorbance is lower than 2.0 and positioned on the linear point of plotted curve.

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 ℃ overnight in a CO_2 incubator to allow the cells to adhere and then replace the medium with 50 µl of fresh medium.
2) Add 50 µl of medium containing test substance that adjusted to the desired concentration (Refer to Table 1).
3) Incubate the plate at 37 ℃ for an appropriate time 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℃ for 30 minutes in a CO_2 incubator.
5) Add 100 µl of the Working Solution to each well. Protect the plate from light and incubate it at the room temperature for 30 minutes.
6) Add 50 µl of the Stop Solution to each well.
7) Measure the absorbance at 490 nm by a microplate reader.

Calculation

Calculation of Cytotoxicity

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

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

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

Table 1 Amount of each solution (Homogeneous assay)

	Test substance	High control	Low control	Background control
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.

General Protocol
Non-homogeneous assay

Optimization of cell concentration

1) Collect cells and wash them with the medium. Prepare cell suspension to 5×10^5 cells/ml in the medium.
2) Add 100 µl of the 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]
   Add the cell suspension (5×10^5 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). Transfer 100 µl from the first well to the next well [B], and mix by pipetting. Repeat this procedure.
4) Add 100 µl of the medium to each well.
5) Incubate the plate at 37 ℃ 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 ℃ 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.
10) Add 100 µl of the Working Solution to each well. Protect the plate from light and incubate it at the 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.
    *Plotting the data by setting absorbance for the x-axis and cell concentration for the y-axis to determine the optimum cell concentraiton as reffering below recomendation.
    - The difference in the absorbance between high control and low control is at least 0.2.
    - The absorbance is lower than 2.0 and positioned on the linear point of plotted curve.

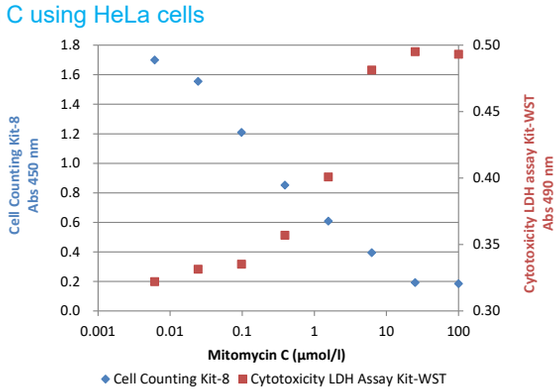
Cytotoxicity Assay

1) Add 100 µl of the cell suspension to each well of a 96-well tissue culture plate.
   * For adherent cells: incubate the plate at 37 ℃ overnight in a CO_2 incubator to allow the cells to adhere and then replace the medium with 100 µl of fresh medium.
2) Add 100 µl of the medium containing test substance that adjusted to the desired concentration (Refer to Table 2).
3) Incubate the plate at 37 ℃ for an appropriate time 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 ℃ 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.

Table 2 Amount of each solution (Non-homogeneous assay)

	Test substance	High control	Low control	Background control
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	-	-

Cytotoxicity of mitomycin C using HeLa cells



Test substance: mitomycin C
Incubation: 37 ℃ , 5% CO_2, 48 hours
Cell line: HeLa
Culture medium: MEM, 10% FBS

Fig. 3 Cytotoxicity of mitomycin C using HeLa cells

If you need more information, please contact Dojindo technical service.

Dojindo Laboratories
2025-5 Tabaru, Mashiki-machi, Kamimashiki-gun, Kumamoto
861-2202, Japan Phone: +81-96-286-1515 Fax: +81-96-286-1525
E-mail: info@dojindo.co.jp Web: www.dojindo.co.jp

Dojindo Molecular Technologies,Inc.
Tel: +1-301-987-2667 Web:http://www.dojindo.com/
Dojindo EU GmbH
Tel: +49-89-3540-4805 Web: http://www.dojindo.eu.com/
Dojindo China Co., Ltd
Tel: +86-21-6427-2302 Web:http://www.dojindo.cn/