

The supplemental manual of this kit is available online. Visit our website, www.dojindo.com, put search keyword "CK12" in the search bar to access the product page for the download.

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.

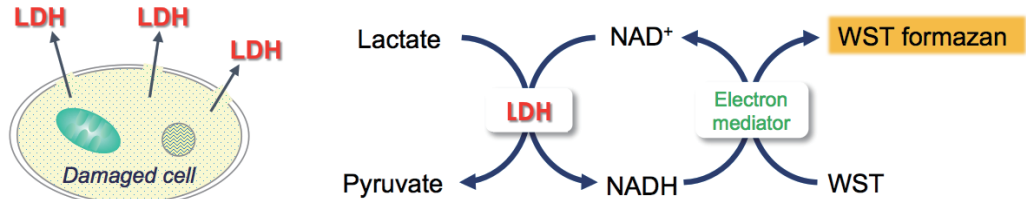
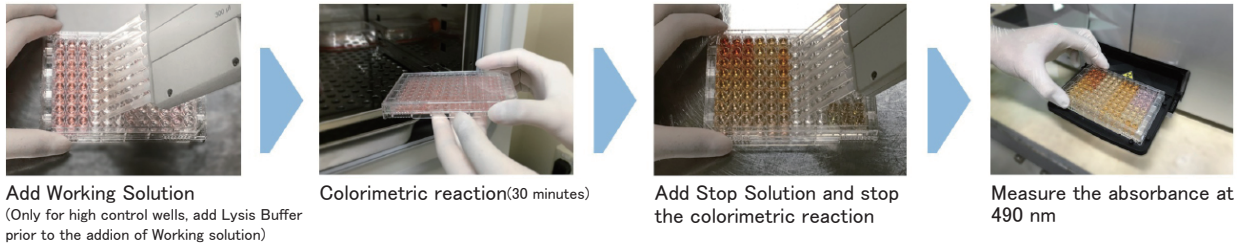


Fig. 1 Principle of cytotoxicity measurement

Procedure



Add Working Solution
(Only for high control wells, add Lysis Buffer prior to the addition 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

- CO₂ incubator
- Microplate reader (490 nm filter)
- 96-well tissue culture plate (flat-bottomed)
- 20, 100-200 µl multichannel pipettes
- 96-well optically clear plate (flat-bottomed)
- *For non-homogeneous assay
- *For suspension cells in non-homogeneous assay: round or V-bottomed plate.

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

- To dissolve the powder, add 1 ml of Assay Buffer for the 100 tests unit or 5 ml of Assay Buffer for 500 tests unit to the Dye Mixture vial. Close the cap and dissolve the contents completely.
- 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.

Optimization of cell concentration

- Collect cells and wash them with the medium. Prepare cell suspension to 5×10^5 cells/ml in the medium.
- Add 100 µl of the medium to each well of a flat-bottom 96-well tissue culture plate.
- 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 100 µl of 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 (2.5×10^5 cells/well). Transfer 100 µl from the first well to the next well [B], and mix by pipetting. Repeat this procedure.
- Incubate the plate at 37 °C for an appropriate time in a CO₂ incubator.
*Use the same incubation time in the cytotoxicity assay.
- 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 CO₂ incubator.
- 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.
- Add 50 µl of the Stop Solution to each well.
- Measure the absorbance at 490 nm by a microplate reader.

*Plot the data by setting cell concentrations on the x-axis and absorbances on the y-axis. The plot should have two linear lines, one representing High Control and another Low Control. Determine the optimum cell concentration based on the followings.

- Eliminate cell concentrations that has absorbances higher than 2.0.
- Choose cell concentrations that has at least 0.2 difference in the absorbance between high control and low control.

General Protocol Homogeneous assay

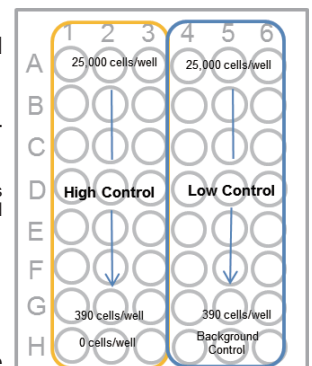


Fig. 2 Plate arrangement

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 CO₂ 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 °C for an appropriate time period in a CO₂ 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 CO₂ 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.

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.

Calculation

Calculation of Cytotoxicity

Calculate the average absorbance from each triplicate set of wells and subtract the background control value from each absorbance one. 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

General Protocol

Optimization of cell concentration

- 1) Collect cells and wash them with the medium. Prepare cell suspension to 5×10⁵ 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 100 µl of the cell suspension (5×10⁵ cells/ml) to the first well [A] and mix by pipetting. This well contains the maximum number of cells (2.5×10⁴ 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 °C for an appropriate time in a CO₂ 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 CO₂ 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.

*Plot the data by setting cell concentrations on the x-axis and absorbances on the y-axis. The plot should have two linear lines, one representing High Control and another Low Control. Determine the optimum cell concentration based on the followings.
- Eliminate cell concentrations that has absorbances higher than 2.0.
- Choose cell concentrations that has at least 0.2 difference in the absorbance between high control and low control.

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 °C overnight in a CO₂ 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 °C for an appropriate time period in a CO₂ 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 CO₂ 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	-	-

Test substance: mitomycin C
Cell line: HeLa
Culture medium: MEM, 10% FBS
Incubation: 37 °C, 5% CO₂, 48 hours

Cytotoxicity of mitomycin C using HeLa cells

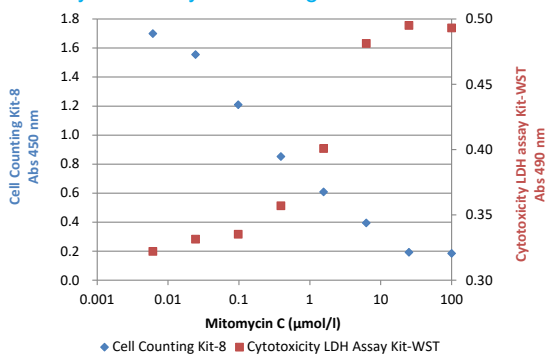


Fig. 3 Cytotoxicity of mitomycin C using HeLa cells

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

Dojindo Molecular Technologies, Inc.

30 West Gude Dr., Suite 260, Rockville, MD 20850, USA

Toll free: 1-877-987-2667 Phone: 301-987-2667 Fax: 301-987-2687

E-mail: info@dojindo.com Web: www.dojindo.com