EZ-LDH

Cell Cytotoxicity Assay Kit

Cat. No. DG-LDH500

DG-LDH1000

FOR RESEARCH USE ONLY.

NOT FOR USE IN DIAGNOSTIC PROCEDURES.

DoGenBio Co., Ltd.

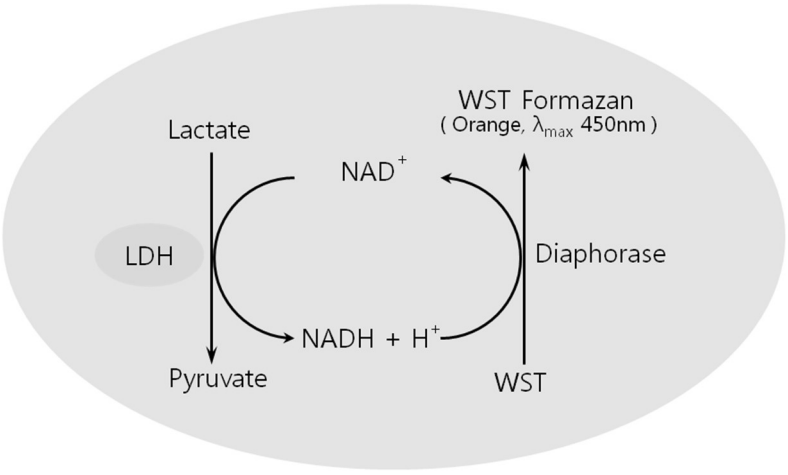
▪ Product Description

This is a kit that can easily measure cytotoxicity/cytolysis by highly sensitively measuring the amount of Lactate dehydrogenase (LDH) released from dead or damaged cells due to apoptosis or necrosis.

Cytotoxicity (Cell death) can be measured indirectly through a cell viability test, but for a more sensitive and accurate measurement, it is recommended to experiment using enzymes related to cell death or cell damage.

Lactate dehydrogenase (LDH) is a stable enzyme that exists in the cytoplasm. Normally, it cannot pass through the cell membrane and is not released out of the cell. However, when the cell membrane is damaged or the cell dies, it is released into the medium. Therefore, the amount of LDH in the medium is proportional to the number of dead or damaged cells.

Based on these characteristics of LDH, the EZ-LDH cytotoxicity assay kit provides a simple cell cytotoxicity test by measuring the amount of LDH released from cells by measuring the absorbance (450 nm) using water soluble tetrazolium salt (WST). This is a product that can be done quickly.



Cell cytotoxicity detection mechanism with EZ-LDH

▪ Kit Contents and Storage Conditions

Component	DG-LDH500	DG-LDH1000	Part Number
	500 assay	1000 assay	
WST Substrate Mix	1 vial (-20℃)	2 vials (-20℃)	DG-LDH S1
LDH Assay Buffer	50 ㎖ (0~4℃)	100㎖ (0~4℃)	DG-LDH B50
Cell Lysis Solution	6 ㎖ (0~4℃)	6㎖ (0~4℃)	-
Stop Solution	6 ㎖ (0~4℃)	6㎖ (0~4℃)	-

* 동결 건조된 WST Substrate는 -20℃에서 6개월간 안정합니다.

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▪ Media

The experiment is conducted using media containing 10% FBS.

If you want to lower the background, you can use serum-free medium or lower the FBS content.

▪ Preparation of Working Solution

Solution	Preparation	Storage and Stability
WST Substrate Mix	After adding 1.1 ml of distilled water to 1 vial, mix for 10 minutes to completely dissolve. ** do not vortex	The dissolved solution is stable for 2 months at -20℃.
Reaction Mixture (Protect from light)	For 100 tests : Mix 10 ml LDH Assay Buffer with 200 ㎕ WST Substrate Mix.	It is recommended to mix the reaction mixture immediately before use.

▪ Preparation of Control

In addition to the preliminary experiment to determine the appropriate number of cells, the experimental method can be broadly divided into two methods: Normal Cytotoxicity assay and Cell mediated Cytotoxicity assay. The control group commonly required for the experiment is as follows.

1) Background control

Measures LDH contained in media's FBS.

2) Low control

During the experiment, the amount of LDH released from naturally dead or damaged cells is measured.

3) High control

The cells used in the experiment are artificially killed by adding 10 ㎕ of Lysis solution, and the maximum amount of LDH that can be released from the cells is measured.

4) Volume control

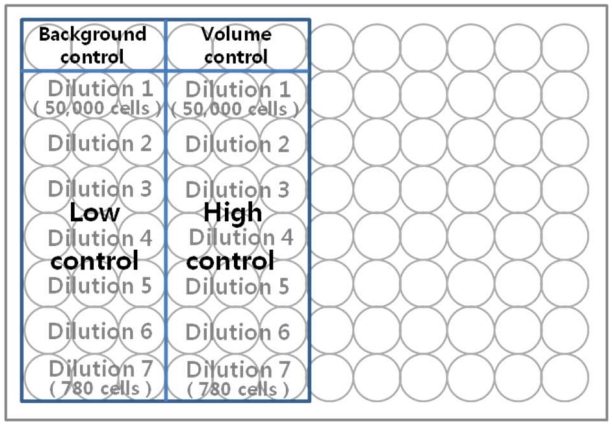
It is measured by adding 10 ㎕ of Lysis solution to 100 ㎕ of Assay media, and is necessary to correct the volume increased by using the lysis solution in High control.

▪ Optimization of Cell number

Since the content of LDH varies depending on the type of cell, it is recommended to determine the optimal cell number through a preliminary experiment to obtain more accurate experimental results. (In the case of cell-mediated cytotoxicity assay, preliminary experiments are performed only on target cells.)

1. Collect the cells in culture and prepare a cell suspension at a number of 5 x 10^4 cells/well.

2. Set 3 Low control and 3 High control controls and serially dilute the prepared cell suspension 1/2 times each. At this time, prepare background control and volume control.



Background control :
Add 100 ㎕ assay media into 3 wells.

Volume Control :
Add 100 ㎕ assay media into 3 wells.

3. Incubate in an incubator for an appropriate time according to the experimental conditions. (e.g. Incubate for the same time as in this experiment, such as 6, 12, 24, and 48 hours.)

4. Add 10 ㎕ of Lysis solution per well to the High control group and Volume control group. (Pipet to ensure lysis or incubate at room temperature for 5 minutes.)

5. Using a centrifuge, collect suspension cells.(600xg, 5 minutes)

6. Take 10 ㎕ of supernatant and transfer it to a new 96 well plate. (Be careful to take only the culture medium and not the cell pellet.)

7. Prepare LDH Reaction Mixture, add 100 ㎕ to each well, and mix carefully.

8. Incubate the plate for 30 minutes* at room temperature blocked from light.
   * Multiple time point measurements are possible, allowing the incubation time to be adjusted. Please adjust the time to obtain an appropriate absorbance value within the range of absorbance Low control OD450 < 0.8, High control OD450 < 2.0.

9. Before measuring the absorbance, shake gently and measure the absorbance at 450 nm using a plate reader. (Reference wavelength : 600~650 nm)

10. The appropriate number of cells is determined by the number of cells that maximizes the difference in absorbance between low control and high control.

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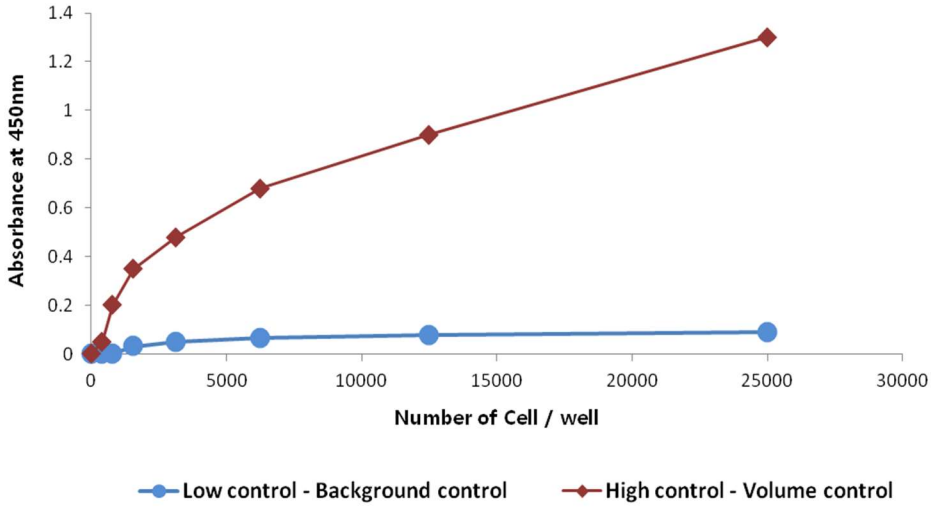
* Example of determining the optimal number of cells

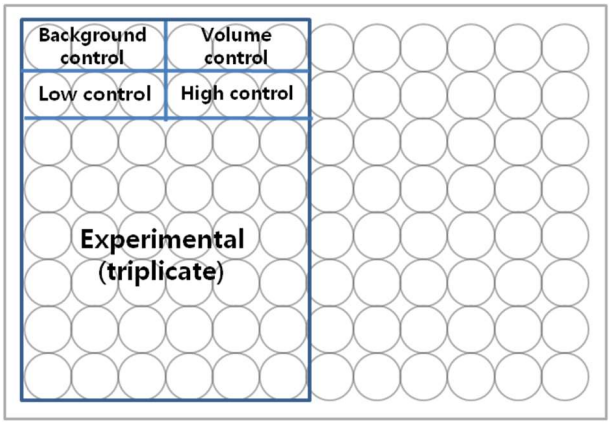


After the experiment according to the method of **Optimization of Cell number**, if the High control and Low control were corrected with Volume control and background control respectively and the following results were obtained, the optimal cell number suitable for the experiment would be the absorbance value. The largest difference is 25,000 cells/well (100 ㎕).

▪ General Protocol – Cell Cytotoxicity Assay

A. Normal Cytotoxicity Protocol

1. **Optimization of Cell number** Prepare a cell suspension at twice the number of the number of cells determined through preliminary experiments, add 50 ㎕ per well into a 96 well plate, and culture in a CO_2 incubator for about 24 hours. (e.g., at 37℃, 5% CO_2)



2. Add 50 ㎕ of test substances prepared at various concentrations to each well.
(At this time, 50 ㎕ of medium was added to each control group to make the total volume 100 ㎕.

3. Incubate in an incubator for an appropriate time according to the experimental conditions.
(e.g. 6, 12, 24, 48 hours)

4. Add 10 ㎕ of Lysis solution per well to the High control group and Volume control group.
(Pipet to ensure lysis or incubate at room temperature for 5 minutes.)

5. Using a centrifuge, collect suspension cells.(600xg, 5 minutes)

6. Take 10 ㎕ of supernatant and transfer it to a new 96 well plate.
(Be careful to take only the culture medium and not the cell pellet.

7. Prepare LDH Reaction Mixture, add 100 ㎕ to each well, and mix carefully.

8. Incubate the plate for 30 minutes* at room temperature blocked from light.
* Multiple time point measurements are possible, allowing the incubation time to be adjusted. Please adjust the time to obtain an appropriate absorbance value within the range of absorbance Low control OD450 < 0.8, High control OD450 < 2.0.

9. Before measuring the absorbance, shake gently and measure the absorbance at 450 nm using a plate reader.(Reference wavelength : 600~650 nm)
* option : The reaction can be stopped by adding 10 ㎕ Stop solution.

* Calculation of cytotoxicity

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

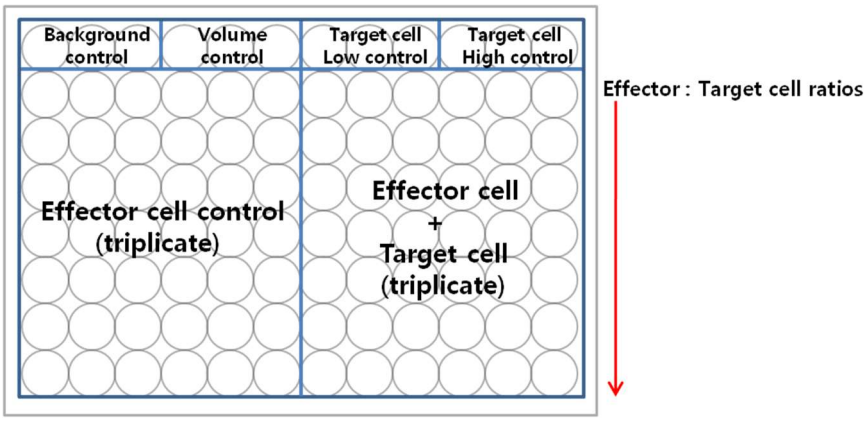
A : Exp. - Background control

B : Low control - Background control

C : High control - Volume control

B. Cell mediated Cytotoxicity Protocol

1. Effector cells (NK cells, CTLs, etc.) are diluted two-fold using measurement medium and added at 50 ㎕ each into a 96-well plate. At this time, an effector cell control group is also set up and prepared to measure LDH naturally released from effector cells.



2. Prepare a cell suspension with twice the number of target cells as determined through the **Optimization of Cell number** preliminary experiment and add 50 ㎕ each to the

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well containing the effector cells. At this time, low control and high control of target cells are created in 3 wells each.
(Add 50 ㎕ of medium to each control group to bring the total volume to 100 ㎕.)

3. Incubate in an incubator for an appropriate time according to the experimental conditions.
(e.g. 6, 12, 24, 48 hours)

4. Add 10 ㎕ of Lysis solution per well to the Target cell High control group and Volume control group. (Pipet to ensure lysis or incubate at room temperature for 5 minutes.)

5. Measure the same as steps 5 to 9 of the **Normal Cytotoxicity Protocol**.

* Calculation of cytotoxicity

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

A : Exp. – Background control
B : Target cell low control – Background control
C : Effector cell control – Background control
D : Target cell high control – volume control

▪ Related Product

	Products	Catalog No.	Assay
Cell Proliferation / Cytotoxicity	EZ-Cytox	EZ-500	500 Assay
		EZ-1000	1000 Assay
		EZ-3000	3000 Assay
		EZ-5000	5000 Assay
		EZ-BULK150	10000 Assay
	EZ-Cytox^{PLUS}	EZ-3000P	3000 Assay
Cell Cytotoxicity	EZ-LDH	DG-LDH500	500 Assay
		DG-LDH1000	1000 Assay