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18250E_1908_1

Product No. 18250

LDH Cytotoxicity Assay Kit

Measurement of lactate dehydrogenase (LDH) released from damaged cells is a widely used assay for quantification of cytotoxicity because it is safer and simpler method compared with radioisotope ^{51}Cr method. LDH is a stable enzyme, present in cytoplasm of living cells, and rapidly released into the cell culture medium upon damage of the plasma membrane (e.g., Necrosis). Therefore, cytotoxicity is quantified by measurement of LDH activity released into the cell culture medium.

Features

- Economical size (100 TESTS/500 TESTS)
- Colorimetric assay using water-soluble tetrazolium salt (WST)
- Simple and easy assay protocol
- Measured by a microplate reader based on absorbance detection method
- One pre-mixed substrate solution (No need to prepare the reagents)
- Can be stored in a refrigerator (Stable for three months)
- Other cell viability assays can be done in combination with this assay by using cell culture supernatant

Components

Reagents	100 TESTS			500 TESTS		
	Volume	Quantity	Bottle	Volume	Quantity	Bottle
Substrate Solution	10 mL	1	Brown	50 mL	1	Brown
Lysis Solution	1 mL	1	White	5 mL	1	White
Stop Solution	5 mL	1	White	25 mL	1	White

Preparation

No need to prepare the reagents. All reagents can be used immediately after thawing at room temperature.

Protocol

The amount of LDH in cytoplasm varies among the cell types. A preliminary experiment to optimize the number of cells per well and absorbance value for each control is recommended for the first time measurement.

1. A Method of adding reagent to cell culture supernatant

Because of using cell culture supernatant, the cells can be used for other assays.

1-1) Determination of optimal cell number per well

(1) Collect cells and wash them with the culture medium.
(2) Prepare the cell suspension of specific cell density in the culture medium (Refer to Table 1).
(3) Add 100 µL of the cell suspension of each cell density to the low and high control wells (triplicate). Refer to Table 2 for the solution volume adding to each well in step (4), (5) and (7) below.
(4) Add 220 µL of the culture medium to three wells (Background control) in addition to (3) *1.
(5) Add 120 µL of the culture medium to each low control well and 100 µL of the culture medium to each high control well.
(6) Incubate the plate in a CO_2 incubator at 37 ˚C *2.
(7) Add 20 µL of the Lysis Solution to each high control well *3.
(8) Incubate the plate for 30 minutes in a CO_2 incubator at 37 ˚C.
(9) Transfer 100 µL of the supernatant from each well to an optically clear 96-well plate *4.
(10) Add 100 µL of the Substrate Solution to each well of the plate above.
(11) Protect the plate from light and incubate it for 20 minutes at room temperature.
(12) Add 50 µL of the Stop Solution to each well.
(13) Measure the absorbance values at 490 nm by a microplate reader *5.

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[*1] Each control is defined as follows.

Background control

The absorbance value of LDH activity derived from serum and phenol red in the culture medium.
The absorbance value of the background control has to be subtracted from the absorbance values of all other wells.

Low control

The absorbance value of LDH activity naturally released from normal cells in the culture medium.

High control

The absorbance value of maximum LDH activity released from the lysed cells.

[*2] Since the living cells grow in culture, use the same incubation time as the cytotoxicity assay.

[*3] After adding the Lysis Solution, tap the corners of the plate so that the Lysis Solution sufficiently diffuses. Cells may not be lysed enough when the diffusion is insufficient.

[*4] In case of suspension cells, centrifuge the micro-plate at 250 - 300 xg for 2 minutes before step (9). V-bottom plate is recommended for suspension cells.

[*5] The optimal cell density for cytotoxicity assay should be selected from the ones showing a linear relationship with the absorbance value of high control, and should be the one produces the greatest differences of the absorbance value between the high and low controls.

Table 1 : Plate arrangement

Cell density (cells/mL)	Low control (Triplicate)			High control (Triplicate)		
$2.50X10^5$	25,000 cells/well			25,000 cells/well		
$1.25X10^5$	12,500 cells/well			12,500 cells/well		
$6.25X10^4$	6,250 cells/well			6,250 cells/well		
$3.13X10^4$	3,125 cells/well			3,125 cells/well		
$1.56X10^4$	1,562 cells/well			1,562 cells/well		
$7.81X10^3$	781 cells/well			781 cells/well		
$3.91X10^3$	390 cells/well			390 cells/well		
-	Background control (Triplicate)					
-	-	-	-	-	-	-

Talbe 2 : Solution volume of each well

	Low control	High control	Background
Cell suspension	100 µL	100 µL	-
Culture medium	120 µL	100 µL	220 µL
Lysis Solution	-	20 µL	-

1-2) Cytotoxicity Assay

(1) Collect cells and wash them with the culture medium.
(2) Adjust the cell suspension to the desired density by using the culture medium.
(3) Add 100 µL of the cell suspension to the sample, low and high control wells (triplicate). Refer to Table 3 for the solution volume adding to each well in step (4), (5), (6) and (8).
(4) Add 220 µL of the culture medium to three wells (Background control) in addition to (3)[*1].
(5) Add 20 µL of the culture medium to each sample well, 120 µL to each low control well and 100 µL to each high control well.
(6) Prepare the culture medium containing test substance that adjusted to the desired concentration. Add 100 µL of this culture medium to each sample well.

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(7) Incubate the plate in a CO_2 incubator at 37 ˚C .
(8) Add 20 µL of the Lysis Solution to each high control well [*3].
(9) Incubate the plate for 30 minutes in a CO_2 incubator at 37 ˚C.
(10) Transfer 100 µL of the supernatant from each well to an optically clear 96-well plate [*4].
(11) Add 100 µL of the Substrate Solution to each well of the plate above.
(12) Protect the plate from light and incubate it for 20 minutes at the room temperature.
(13) Add 50 µL of the Stop Solution to each well.
(14) Measure the absorbance at 490 nm by a microplate reader.
(15) Determine the percentage cytotoxicity by the following equation.
Calculate the average absorbance values of triplicate samples and controls, and then subtract the absorbance value of the background control from the absorbance values of each well.

$$\text{Cytotoxicity (\%)} = \frac{\text{(Test substance – Low control)}}{\text{(High control – Low control)}} \times 100$$

Table 3 : Solution volume of each well

	Sample	Low control	High control	Background
Cell suspension	100 µL	100 µL	100 µL	-
Culture medium	20 µL	120 µL	100 µL	220 µL
Test substance in culture medium	100 µL	-	-	-
Lysis Solution	-	-	20 µL	-

2. A method of adding reagent directly to the cell culture medium

In this method, cytotoxicity can be determined by adding reagent directly to the cell culture medium in which living cells and damaged cells are mixed.

2-1) Determination of optimal cell number per well

(1) Collect cells and wash them with the culture medium.
(2) Prepare the cell suspension of specific cell density in the culture medium (Refer to Table 1).
(3) Add 100 µL of the cell suspension of each cell density to the low and high control wells (triplicate). In the following steps, refer to Table 4 for the solution volume adding to each well.
(4) Add 100 µL of the medium to three wells (Background control) in addition to (3)[*1].
(5) Incubate the plate in a CO_2 incubator at 37 ˚C [*2].
(6) Add 10 µL of the Lysis Solution to each high control well [*3].
(7) Incubate the plate for 30 minutes in a CO_2 incubator at 37 ˚C.
(8) Add 100 µL of the Substrate Solution to each well.
(9) Protect the plate from light and incubate it for 20 minutes at the room temperature.
(10) Add 50 µL of the Stop Solution to each well.
(11) Measure the absorbance values at 490 nm by a microplate reader [*5].

Table 4 : Solution volume of each well

	Low control	High control	Background
Cell suspension	100 µL	100 µL	-
Medium	-	-	100 µL
Lysis Solution	-	10 µL	-
Substrate Solution	100 µL	100 µL	100 µL
Stop Solution	50 µL	50 µL	50 µL

2-2) Cytotoxicity Assay

(1) Collect cells and wash them with the culture medium.
(2) Adjust the cell suspension to the desired density by using the culture medium.
*In this method, 50 µL of the cell suspension is added to each well. Therefore, prepare the cell density two-fold higher than the optimal cell density determined in 2-1) above.
(3) Add 50 µL of the cell suspension to the sample, low and high control wells (triplicate). In the following steps, refer to Table 5 for the solution volume adding to each well.
(4) Add 100 µL of the culture medium to three wells (Background control) in addition to (3)[*1].
(5) Add 50 µL of the culture medium to each low and high control well.

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(6) Prepare the culture medium containing test substance that adjusted to the desired concentration. Add 50 µL of this culture medium to each sample well.
(7) Incubate the plate in a CO_2 incubator at 37 ˚C .
(8) Add 10 µL of the Lysis Solution to each high control well [*3].
(9) Incubate the plate for 30 minutes in a CO_2 incubator at 37 ˚C.
(10) Add 100 µL of the Substrate Solution to each well.
(11) Protect the plate from light and incubate it for 20 minutes at the room temperature.
(12) Add 50 µL of the Stop Solution to each well.
(13) Measure the absorbance values at 490 nm by a microplate reader .
(14) Determine the cytotoxicity by using the same equation in step 1-2) above.

Table 5 : Solution volume of each well

	Sample	Low control	High control	Background
Cell suspension	50µL	50µL	50µL	-
Culture medium	-	50µL	50µL	100µL
Test substance in culture media	50µL	-	-	-
Lysis Solution	-	-	10µL	-
Substrate Solution	100µL	100µL	100µL	100µL
Stop Solution	50µL	50µL	50µL	50µL

Caution

High background may be caused by the LDH from serum in the medium. In this case, use the medium in which the serum concentration is reduced to about 1%.

Storage

-20 C, Protect from light
*Stable for three months in a refrigerator.

Expiration

Expiration date is stated on the product label.

Packing

100 TESTS (Product No.18250-64)
500 TESTS (Product No.18250-35)

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