Product Information

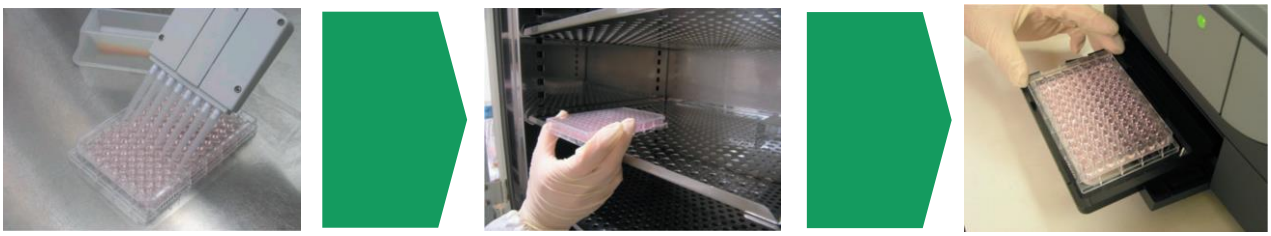
91963 Cytotoxicity LDH Assay Kit-WST

Storage temperature: -20°C. Protect from light.

General information
Lactate dehydrogenase (LDH), present in all types of cells, is one of the most stable enzymes on the outside of cells (in the medium). Therefore, measurement of released LDH from the cytoplasm into the surrounding culture medium has been widely accepted to determine cytotoxicity. Cytotoxicity LDH Assay Kit-WST allows to measure LDH released from damaged membranes by utilizing a water-soluble tetrazolium salt (WST). In addition, it enables both homogeneous and non-homogeneous assay.

Features
- Simple colorimetric assay using a water-soluble tetrazolium salt (WST)
- Applicable for high-throughput screening using a microplate reader
- Available for both homogeneous and non-homogeneous assay
- Ready-to-use solutions (no-premixing and no reconstitution required)

Procedure



Add Reagent solution to high control wells

Incubate cells for 20 min

Measure absorbance at 490 nm

Kit contents (100 tests)
- 1x 10 ml Reagent solution
- 1x 1 ml Lysis Solution
- 1x 5 ml Stop Solution

Precaution
LDH contained in the serum may cause a high background value. Adjust serum content 1% or less as a final concentration.

How to use this product
The amount of intracellular LDH depends on type of cells. Therefore, the number of cells used cytotoxicity assay should be optimized by measuring an absorbance of High control, Low control and Background control.
- High control: LDH activity in the cells (the cells have to be lysed)
- Low control: The spontaneous released LDH activity in the supernatant.
- Background control: LDH activity in the medium

1. Application 1: Non-homogeneous assay (without cells)
   Since only culture supernatant is used for non-homogeneous assay, the remaining cells can be applied for other experiments such as cell viability assay, cell staining, immunostaining etc.

   1.1. : Optimization of Cell Concentration
   - 1.1.1. Wash the cells with a medium
   - 1.1.2. Make serial 2-fold dilution of the cells as shown in Figure 1
   - 1.1.3. Add the medium to each well as described in Table 1
   - 1.1.4. Incubate the plate at 37°C in a CO_2 incubator[1]
   - 1.1.5. Add 20 µl of Lysis Solution to each well of High control[2]
   - 1.1.6. Incubate the plate at 37°C in a CO_2 incubator for 30 minutes
   - 1.1.7. Transfer 100 µl of supernatant of each well to a microplate[3]
   - 1.1.8. Add 100 µl of Reagent Solution to each well
   - 1.1.9. Incubate the cells at room temperature under protection from light for up to 20 minutes
   - 1.1.10. Add 50 µl of Stop Solution to each well
   - 1.1.11. Measure the absorbance at 490 nm by using a microplate reader. To minimize an experimental error, and reproducibility, use a cell concentration that shows the largest OD difference between High control and Low control.

   Description of controls
   Background Control: LDH activity in the medium. The value of background control is subtracted from values of High control and Low control.
   Low Control: The spontaneous released LDH activity in the supernatant.
   High Control: Maximum LDH activity in the cells by adding of Lysis Solution
   [1] Adjust incubation time according to the exposure time of test substance to cells.
   [2] After adding Lysis Solution, tap the plate gently to mix the cells and lysis solution thoroughly.
   [3] For suspension cells, V-bottomed plate is recommended for the assay and centrifuge the microplate (250~300 × *g*, for 2 minutes) before proceeding to step 1.1.7.

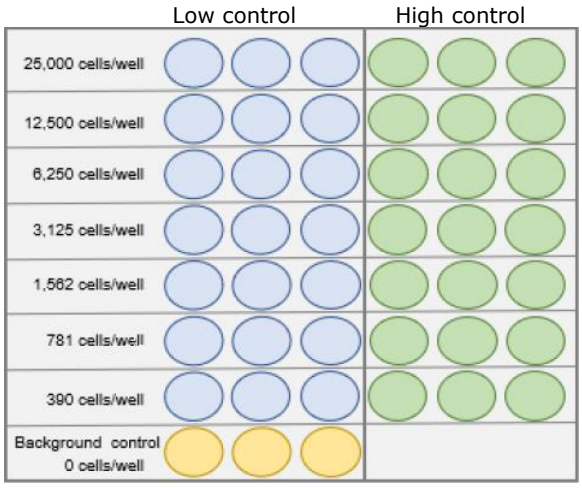


Figure 1: Example of plate arrangement

Table 1: The amount of solution to be added to each well

	Background control	Low control	High control
Cell suspension	-	100 µl	100 µl
Medium	220 µl	120 µl	100 µl
Lysis Solution	-	-	20 µl

1.2 Cytotoxicity Assay

1.2.1. Wash the cells with the medium
1.2.2. Prepare a cell suspension to be the optimized cell concentration in step 1.1.11.
1.2.3. Add 100 µl of cell suspension to each well (triplicates). Refer to the Table 2
1.2.4. Add the medium to each well as described in the Table 2
1.2.5. Add 100 µl of test substance to each sample well (Experimental sample)
1.2.6. Incubate the plate at 37°C in a CO_2 incubator for an appropriate incubation time
1.2.7. Add 20 µl of Lysis Solution to each well of High control[1]
1.2.8. Incubate the cells at 37°C in a CO_2 incubator for 30 minutes
1.2.9. Transfer 100 µl of supernatant of each well to a microplate[2]
1.2.10. Add 100 µl of Reagent Solution to each well
1.2.11. Incubate the cells at room temperature under protection from light for up to 20 minutes
1.2.12. Add 50 µl of Stop Solution to each well
1.2.13. Measure the absorbance at 490 nm by using a microplate reader
1.2.14. Calculate a percentage of cytotoxicity with the following equation

[1] After adding Lysis Solution, tap the plate gently to mix the cells and lysis solution thoroughly.
[2] For suspension cells, V-bottomed plate is recommended for the assay and centrifuge the microplate (250～300 × g, for 2 minutes) before proceeding to step 1.2.10.

$$Cytotoxicity\ (\%) = \frac{(Experimental\ sample - Background\ control) - (Low\ control - Background\ control)}{(High\ control - Background\ control) - (Low\ control - Background\ control)} \times 100$$

Table 2: The amount of solution to be added to each well

	Background control	Experimental sample	Low control	High control
Cell suspension	-	100 µl	100 µl	100 µl
Medium	220 µl	20 µl	120 µl	100 µl
Test substance		100 µl		
Lysis Solution	-	-	-	20 µl

2. Application 2: Homogeneous Assay

Reagent solution is added to a cell culture medium. There is no need to transfer cell culture supernatant to a new microplate for the assay.

2.1. Optimization of Cell Concentration

2.1.1. Wash the cells with the medium
2.1.2. Make serial 2-fold dilution of the cells as shown in the Figure 1
2.1.3. Add the medium to each well as described in the Table 3
2.1.4. Incubate the plate at 37°C in a CO2 incubator[1]
2.1.5. Add 10 µl of Lysis Solution to each well of High control[2]
2.1.6. Incubate the plate at 37°C in a CO2 incubator for 30 minutes
2.1.7. Add 100 µl of Reagent Solution to each well
2.1.8. Incubate the cells at room temperature under protection from light for up to 20 minutes
2.1.9. Add 50 µl of Stop Solution to each well
2.1.10. Measure the absorbance at 490 nm by using a microplate reader.
To minimize an experimental error, and reproducibility, use a cell concentration that shows the largest OD difference between High control and Low control.

Description of controls

Background Control: LDH activity in the medium. The value of background control is subtracted from values of High control and Low control.
Low Control: The spontaneous released LDH activity in the supernatant
High Control: Maximum LDH activity in the cells by adding of Lysis Solution
[1] Adjust incubation time according to the exposure time of test substance to cells
[2] After adding Lysis Solution, tap the plate gently to mix the cells and lysis solution thoroughly

Table 3: The amount of solution to be added to each well

	Background control	Low control	High control
Cell suspension	-	100 µl	100 µl
Medium	100 µl	-	-
Lysis Solution	-	-	10 µl
Reagent Solution	100 µl	100 µl	100 µl
Stop Solution	50 µl	50 µl	50 µl

2.2. Cytotoxicity Assay

2.2.1. Wash the cells with the medium

2.2.2. Prepare a cell suspension to be the optimized cell number in a well.
For homogeneous assay, 50 µl of cell suspension is used. Therefore, double the concentration of cells which is determined in step 2.1.10.

2.2.3. Add 50 µl of cell suspension to each well (triplicates). Refer to Table 4

2.2.4. Add the medium to each well as described in Table 4

2.2.5. Add 100 µl of test substance to each sample well (Experimental sample)

2.2.6. Incubate the plate at 37°C in a CO_2 incubator for an appropriate incubation time

2.2.7. Add 10 µl of Lysis Solution to each well of High control[1]

2.2.8. Incubate the cells at 37°C in a CO_2 incubator for 30 minutes

2.2.9. Add 100 µl of Reagent Solution to each well

2.2.10. Incubate the cells at room temperature under protection from light for up to 20 minutes

2.2.11. Add 50 µl of Stop Solution to each well

2.2.12. Measure the absorbance at 490 nm by using a microplate reader

2.2.13. Calculate a percentage of cytotoxicity with the following equation

[1] After adding Lysis Solution, tap the plate gently to mix the cells and lysis solution thoroughly

$$Cytotoxicity\ (\%) = \frac{(Experimental\ sample - Background\ control) - (Low\ control - Background\ control)}{(High\ control - Background\ control) - (Low\ control - Background\ control)} \times 100$$

Table 4: The amount of solution to be added to each well

	Background control	Experimental sample	Low control	High control
Cell suspension	-	50 µl	50 µl	50 µl
Medium	100 µl	-	50 µl	50 µl
Test substance	-	50 µl	-	-
Lysis Solution	-	-	-	10 µl
Reagent Solution	100 µl	100 µl	100 µl	100 µl
Stop Solution	50 µl	50 µl	50 µl	50 µl

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.