Cat. # MK401

For Research Use

TaKaRa

LDH Cytotoxicity Detection Kit

Product Manual

v201906

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I. Description

Cell death is often assayed by the quantification of plasma membrane damage. Several standard methods for quantification of cellular viability were developed to provide sensitive, reliable, and automated methods for quantifying cell death.

Widely used cell viability and toxicity analysis methods are often based on the uptake or exclusion of dyes such as trypan blue or eosin Y. These methods have several disadvantages, including the inability to process large numbers of samples and to quantitate dead cells that have been damaged.[1-4)]

Other analysis methods are based on the measurement of released radioactive isotope or fluorescent dye from pre-labeled, target cells.[5-8)] The disadvantages with these methods are that the target cells must be pre-labeled prior to the assay. Also, cells spontaneously release the labels, generating a background that lowers the sensitivity of the assay.

Some analysis methods are based on the measurement of cytoplasmic enzyme activity released from damaged cells.[9-12)] The amount of enzymatic activity is proportionally correlated with the number of damaged cells. Alkaline and acid phosphatase, glutamate oxaloacetate transaminase (GOT), and glutamate pyruvate transaminase (GPT) have all been used to measure cytotoxicity. However, these enzymes are present in very low amounts in cells, necessitating the use of complex kinetics assays.

The LDH Cytotoxicity Detection Kit uses LDH, a stable cytoplasmic enzyme that is present in large amounts in most cells. LDH is released into the cell culture supernatant during cytoplasmic membrane damage and can be easily measured using standard reagents.

II. Principle

The LDH Cytoxicity Detection Kit provides a colorimetric measure of cell cytotoxicity/cytolysis based on the measurement of LDH activity in the cell culture supernatant. This assay is a non-radioactive alternative to [^{3}H]-thymidine and [^{51}Cr]-release assays. The cell culture supernatant is collected and incubated with the kit's reaction mixture. LDH activity is determined via the two-step enzymatic reaction shown below (Figure 1).

Step 1: NAD^{+} is reduced to NADH/H^{+} by the LDH-catalyzed conversion of lactate to pyruvate.

Step 2: Diaphorase, a catalyst, transfers H/H^{+} from NADH/H^{+} to the yellow tetrazolium salt INT, which becomes reduced to a red formazan product.

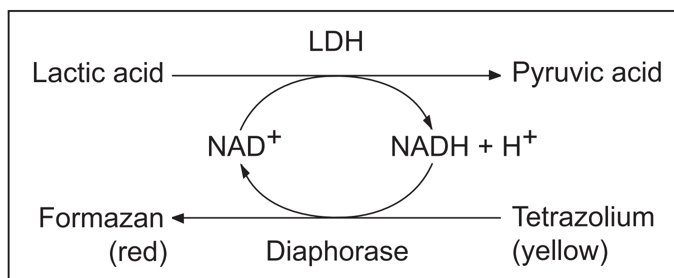


Figure 1. Principle of measurement

An increase in the number of dead or plasma membrane-damaged cells results in an increase in the total LDH enzymatic activity in the cell culture supernatant. This increase in LDH activity directly correlates with the amount of formazan product that is formed. In essence, the amount of color generated by the assay is directly proportional to the number of damaged cells. The formazan dye itself is water soluble and has a maximal absorption at ~500 nm, whereas the tetrazolium salt INT does not exhibit any significant absorption at this wavelength (Figure 2).

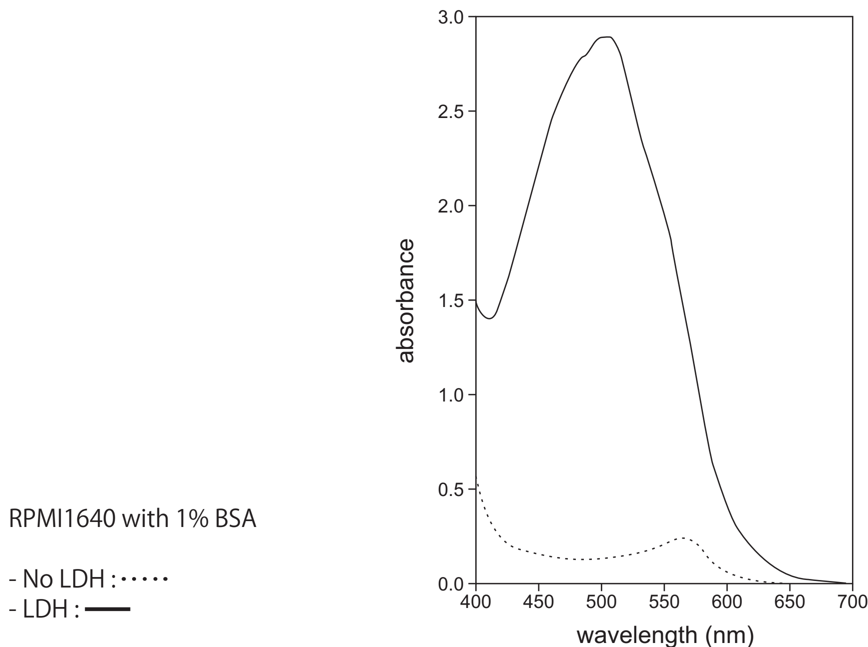


Figure 2. Absorbance spectra of the working solutions of LDH Cytotoxicity Detection Kit

This kit can be used in various *in vitro* cell systems to measure damage to the plasma membrane.

- Detection and quantification of cell-mediated cytotoxicity induced by cytotoxic T-lymphocytes (CTL), natural killer (NK) cells, lymphokine activated killer (LAK) cells or monocytes[12,13)]
- Determination of factors that lead to cytolysis[12)]
- Measurement of antibody-dependent cellular cytotoxicity (ADCC) and complement-mediated cytolysis
- Determination of the cytotoxic potential of compounds in environmental and medical research and in the food, cosmetic, and pharmaceutical manufacturing[14-21)]
- Determination of cell death in bioreactors[22-24)]

It has been confirmed that precise evaluation of cell death during termination in bioreactors could be performed by measuring the release of cytoplasmic LDH enzyme into the culture medium. Moreover, a good correlation has been confirmed between LDH-release assay and the [^{51}C] release assay in cell-mediated cytotoxicity studies using a variety of murine and human effector-target cell systems, including NK cells, CTL, and macrophages as effector cells.

III. Features

1) Safe :	No radioactive isotopes are required.
2) Accurate :	Assay results obtained with this kit strongly correlate to the number of damaged cell.
3) High sensitivity :	Low cell numbers (0.2 - 2 x 10^4 cells/well) can be used for detection.
4) Fast :	A large number of samples can be processed simultaneously using a multiwell-ELISA reader. Only 0.5 - 1 hours are required for measurement.
5) Simple procedure :	- No need for prelabeling and washing steps. - As this kit does not require radioactive isotopes, disposal and radiation safety procedures/paperwork are not required.
6) Guaranteed performance :	Performance is tested with every lot.

IV. Components (for 2,000 tests)

- Bottle 1 (blue cap) x 5 : Catalyst (Diaphorase/NAD+), lyophilized
- Bottle 2 (red cap) x 5 : Dye solution (INT/Na-lactate), 45 ml

＊ containing iodotetrazolium chloride (INT) and sodium lactate

V. Storage- -20℃

＊ Lyophilized Catalyst can be stored for one year at -20℃, or for several weeks at 4℃.
Dissolved Catalyst can be stored for several weeks at 4℃.
Thawed Dye solution can be stored for several weeks at 4℃.

VI. Preparation of Solutions

VI-1. Precautions

1) Assay background

Assay background may increase due to the following conditions and should be accounted for by using appropriate controls:

- LDH activity in serum or assay substances. (See VI-2, VI-4)
- When assaying cell-mediated cytotoxicity, the amount of LDH released from damaged effector cells may influence the assay results. (See VI-4, VII-2)
- Substances which interfere LDH or diaphorase enzyme activity influence assays. (See VI-4)

2) Sample handling

Cell-free culture supernatant : Remove cells from the culture medium by centrifugation at ~250*g* prior to assay. Prepared culture supernatant can be stored at 4℃ for a few days without loss of LDH activity.

VI-2. Materials Required but not Provided

Equipment

- 37℃ incubator
- Centrifuge with rotor for microtiter plates
- Microtiter plate (ELISA) reader with 490 - 492 nm filter (A filter over 600 nm is required if a reference wavelength is to be subtracted.)
- Microscope

- Hemocytometer
- Multichannel pipettor (100 μl)
- Sterilized pipette tips
- 96-well microtiter plates (MTP)

  For measurement of cell mediated lysis and analysis of cytotoxic compounds : sterilized, cell-culture grade dishes
  - ・For suspension cells - round or V-bottom
  - ・For adherent cells - flat bottom

  For color development assays - optically clear flat-bottomed

Reagents

- Assay medium

  It is recommended to conduct the assay in the presence of low serum (e.g., 1%) or 1% bovine serum albumin (BSA) (w/v). Both human and animal sera contain various amounts of LDH, which may increase background absorbance in the assay.

- Triton X-100 solution (prepare to 2% Triton X-100 in assay medium)

  Maximal LDH enzyme activity that can be released can be determined by adding a final concentration of 1% Triton X-100 to cell media. LDH activity is not affected by up to 1% Triton X-100.

- HCl stop solution (1 N)

  The reaction product can be measured without adding a stop solution. However, the enzyme reaction can be stopped by the addition of 1 N HCl at 50 μl/well (final concentration : 0.2 N HCl)

- LDH standard preparation

  If the released LDH-activity is to be calculated in unit/ml instead of relative cytotoxicity (%) or absorbance, it is recommended to prepare a standard curve using an appropriate LDH standard solution.

  Please note that assay medium, lysis, and stop solutions and LDH standards are not included in this kit. All other reagents required to perform 2,000 tests are included.

VI-3. Preparation of Reaction Mixtures

1. Solution A : Catalyst (blue cap)

   Reconstitute in 1 ml of distilled water and mix thoroughly for 10 min. The reconstituted solution can be stored for several weeks at 4℃.
2. Solution B : Dye Solution (red cap)

   Thawed INT dye solution is ready to use in the assay. Once thawed, the dye solution can be stored for several weeks at 4℃.
3. Solution C : Reaction mixture＊

   For 100 tests : Prior to use, mix 250 μl of Solution A with 11.25 ml of Solution B.

   For 400 tests : Prior to use, mix the total volume of Solution A with the total volume of Solution B (45 ml).

＊ The reaction mixture must be prepared immediately before use because the reaction mixture can not be stored.

VI-4. Preparation of Controls

The following three controls should be performed in each experiment to calculate percent cytotoxicity. (See Table 1)

1. Background control
   Measure the LDH activity in the assay medium. The absorbance value obtained in this control are subtracted from all other values.
2. Low control
   Measure the spontaneous LDH release from untreated normal cells.
3. High control
   Measure the maximum releasable LDH in the cells by the addition of Triton X-100.

The following two controls are optional :

4. Substance control I
   Measure LDH activity in the test substance. If cell-mediated cytotoxicity is measured, this control provides information on the LDH activity released from the effector cells. (See VII-2)
5. Substance control II
   Determine whether the test substance interferes with LDH activity. Follow the procedures below to perform this control.
   - Add 50 μl/well test substance solution (diluted in assay medium) in triplicate to an optically clear 96-well flat bottom plate.
   - Add 50 μl/well LDH solution (0.05 unit/ml).
   - Add 100 μl/well Solution C (reaction mixture) (see step VI-3) and measure absorbance using a plate reader. Compare the measured absorbance values with values obtained from the control sample (prepare by mixing 50 μl/well LDH solution (0.05 units/ml), 50 μl/well assay medium and 100 μl/ml reaction mixture).

Table 1. Overview of controls

Well contents	Background control*2	Low control*2	High control*2	Substance control I	Substance control II	Experimental setup
Assay medium	200 μl	100 μl	—	100 μl	(50 μl)	—
Cells	—	100 μl	100 μl	—	—	100 μl
Triton X-100 solution*1	—	—	100 μl	—	—	—
Test substance of effector cells	—	—	—	100 μl	50 μl	100 μl
LDH standard	—	—	—	—	50 μl	—

* 1 Triton X-100 solution prepared 2% in the assay medium.
* 2 Background, low, and high controls should be determined for each experiment.

VI-5. Calculation of Cytotoxicity

・Calculation of cytotoxicity

The cytotoxicity (%) is determined by calculating the average absorbance values of the triplicates and subtracting their value from the absorbance value obtained in the background control. The resulting values are used in the following equation:

$$\text{Cytotoxicity (\%)} = \frac{\text{exp. value - low control}}{\text{high control - low control}} \times 100$$

・Calculation of cell-mediated cytotoxicity

The cell-mediated cytotoxicity (%) is determined by calculating the average absorbance of the triplicates and subtracting the background. The resulting values are used in the following equation:

$$\text{Cytotoxicity (\%)} = \frac{\text{A - low control}}{\text{high control - low control}} \times 100$$

A : [effector - target cell mix] - [effector cell control]

VI-6. Preliminary Experiments

(Determining the optimal cell concentration of target cells)
LDH quantity differs depending on the cell type. Therefore, the optimal cell concentration for a specific cell type should be determined in a preliminary experiment. In general, the appropriate cell concentration is that which the difference between the low and high control is maximal. This concentration should be used for the subsequent assays. For most cell lines, the optimal cell concentration is between 0.5 - 2 x 10^4 cells/well in 200 μl (0.25 - 1 x 10^5 cells/ml).

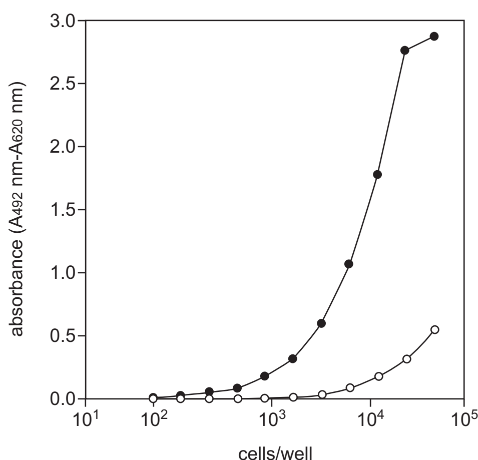
Assay procedure :

1. Fill each wells of a 96-well tissue culture plate with 100 μl assay medium.
2. After washing cells with assay medium, adjust to a concentration of 2 x 10^6 cell/ml with assay medium.
3. Titrate the cells by two-fold serial dilutions across the plate using a multichannel pipette (Table 2). Titrate 100 μl/well of the cell suspension with a micropipettor into B1-B3 (B7-B9) wells containing 100 μl/well assay medium and mix (dilution 1). Transfer 100 μl of the diluted cell suspension from these wells into C1-C3 (C7-C9) wells containing 100 μl/well assay medium (dilution 2). Repeat this step 14 times (to dilution 14).
4. Prepare the following controls on the plate.
   1) Determine the background control : Add 200 μl of assay medium to three wells (A1-A3, Table 2).
   2) Determine the low control (spontaneous LDH release) : Add 100 μl assay medium to three wells containing 100 μl of cells (1-6 of B-H, Table 2).
   3) Determine the high control (maximum LDH release) : Add 100 μl of Triton X-100 solution to three wells containing 100 μl of cells (B-H 7-12, Table 2).
5. Incubate the cells in an incubator (37℃, 5% CO_2, 90% humidity) for the amount of time necessary to assay the test substances.
6. Centrifuge the microtiter plate at 250*g* for 10 min.
7. Remove 100 μl/well supernatant carefully (do not disturb the cell pellet), transfer into the corresponding wells of an optically clear 96-well flat bottom microtiter plate.

8. To determine the LDH activity in these supernatants, add 100 μl of reaction mixture to each well and incubate at room temperature for 30 min. During this incubation period, the plate should be protected from light.
9. Measure the absorbance of the samples at 490 or 492 nm according to the filters available using a platereader. The reference wavelength should be more than 600 nm.

Table 2. Experimental setup to determine the optimal target cell concentration. All tests should be performed in triplicate.

	1 2 3	4 5 6	7 8 9	10 11 12
A	Background control			
B	Cell suspension Dilution 1	Dilution 8	Cell suspension Dilution 1	Dilution 8
C	Dilution 2	Dilution 9	Dilution 2	Dilution 9
D	Dilution 3	Dilution 10	Dilution 3	Dilution 10
E	Dilution 4	Dilution 11	Dilution 4	Dilution 11
F	Dilution 5	Dilution 12	Dilution 5	Dilution 12
G	Dilution 6	Dilution 13	Dilution 6	Dilution 13
H	Dilution 7	Dilution 14	Dilution 7	Dilution 14
	Low control		High control	



K562 cells were titrated in microtiter plates at cell concentration indicated in the X-axis in the figure.
Culture medium (○) was added to determine the spontaneous release of LDH activity and Triton X-100 (●) was added to a final concentration of 1% for the determination of maximum release of LDH activity. Optimal target cell concentration in this experiment is ~1 x 10^4 cells/well.

Figure 3. Determination of the optimal K562 cell concentration.

VII. Protocol

Overview of protocol

Step	Procedure	Volume/well	Time/Temperature
1	Incubate target cells with test substance or cytotoxic effector cells.	200 μl	4 - 24 hrs at 37℃
2	Centrifuge the plate containing cells.		10 min, 250*g* at RT
3	Transfer cell-free culture supernatant to clear, flat bottom microtiterplate.	100 μl	
4	Add Solution C (reaction mixture) and incubate protected from light.	100 μl	~10 - 30 min at RT
5	To stop the reaction, add 1 N HCl to each well.	50 μl	
6	Measure absorbance at about 490 nm. (reference wave length >600 nm)		

RT : room temperature

VII-1. Measurement of the Cytotoxic Potential of Soluble Substances

1. Assay procedure for suspension cells

1. Titrate test substances (mediators, cytolytic, or cytotoxic agents) in the appropriate assay medium in sterile 96-well tissue culture plates by serial dilutions to a final volume of 100 μl/well. All tests should be performed in triplicate (see Table 3).
2. After washing the cells in assay medium, dilute to the optimal concentration determined in the preliminary experiment (VI-6).
3. Add 100 μl/well of cell suspension at the optimal concentration into the dilutions of the test substances.
4. Prepare the following controls on the plate (Table 3)
   1) Background control : 200 μl of assay medium in three wells.
   2) Low control (spontaneous LDH release) : Add 100 μl of cell suspension to three wells containing 100 μl of assay medium.
   3) High control (maximum LDH release) : Add 100 μl of suspension to three wells containing 100 μl of Triton X-100 solution.
   4) Substance control I : Add 100 μl of test substance (with maximum concentration used in the experiment) to three wells containing 100 μl/well of assay medium.
5. Incubate the cells in an incubator (at 37℃, 5% CO_2, 90% humidity) for the time appropriate for the test substances (2 - 24 hrs).
6. After incubation, centrifuge the microtiter plate at 250*g* for 10 min.
7. Carefully remove 100 μl of supernatant from each well (do not disturb the cell pellet) and transfer into corresponding wells of an optically clear 96-well flat bottom microtiter plate.
8. Add 100 μl Solution C (reaction mixture) to each well and incubate for up to 30 min at room temperature. During this incubation period, the plate should be protected from light.
9. Measure the absorbance of the samples at 490 - 492 nm according to the available filters using a plate reader. The reference wavelength should be more than 600 nm.
10. Calculate the percentage cytotoxicity (see VI-5).

Table 3. Experimental setup to measure cell cytotoxicity of substance I and substance II
(All tests should be performed in triplicate.)

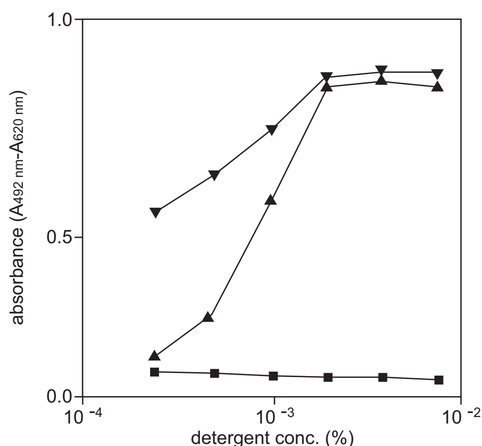
	1–3	4–6	7–9	10–12
A	Background control	Substance control I (Diluton 1)	Substance control II (Diluton 1)	
B	Test substance 1 Dilution 1	Test substance 1 Dilution 8	Test substance 2 Dilution 1	Test substance 2 Dilution 8
C	Dilution 2	Dilution 9	Dilution 2	Dilution 9
D	Dilution 3	Dilution 10	Dilution 3	Dilution 10
E	Dilution 4	Dilution 11	Dilution 4	Dilution 11
F	Dilution 5	Dilution 12	Dilution 5	Dilution 12
G	Dilution 6	Dilution 13	Dilution 6	Dilution 13
H	Dilution 7	Low control	Dilution 7	High control

Two-fold dilution series of test substance 1 + cell suspension (columns 1–6)
Two-fold dilution series of test substance 2 + cell suspension (columns 7–12)

2. Assay procedure for adherent cells

1. After washing the cells in assay medium, dilute to the optimal concentration determined in the preliminary experiment. Add 100 μl of cell suspension per well of a sterile 96-well tissue culture plate. Do not add cells into wells for background control and substance control I.
2. Incubate the cells overnight in an incubator (37℃, 5% CO_2, 90% humidity) to allow the cells to adhere.
3. Immediately before use, titrate the test substances (mediators, cytolytic, or cytotoxic agents) in the appropriate assay medium in a separate microtiter plate by serial dilutions (final volume of 200 μl/well).
4. Remove the assay medium from the adherent cells (removes LDH activity released from the cells during the overnight incubation). Add 100 μl fresh assay medium to each well.
5. Transfer 100 μl of the test substance dilutions into the appropriate wells containing the adherent cells.
6. Prepare the following controls on the plate. (See Table 3)
   1) Background control : Add 200 μl assay medium to three wells.
   2) Low control (spontaneous LDH release) : Add 100 μl assay medium to three wells containing 100 μl of cells.
   3) High control (= maximum LDH release) : Add 100 μl of Triton X-100 solution to triplicate wells containing 100 μl of cells.
   4) Substance control I : Add 100 μl of test substance (maximum concentration used in the experiment) to three wells containing 100 μl of assay medium.

7. Incubate the cells in an incubator (37℃, 5% CO_2, 90% humidity) for the time appropriate for assaying the test substances (2 - 24 hrs, depending on the experimental setup).
8. After incubation, centrifuge the microtiter plate at 250*g* for 10 min.
9. Carefully remove 100 μl/well supernatant (do not disturb the cell pellet) and transfer into corresponding wells of an optically clear 96-well flat bottom microtiter plate.
10. Add 100 μl Solution C (reaction mixture) to each well and incubate for up to 30 min at room temperature to determine the LDH activity in the supernatant. During this incubation period, protect the plate from light.
11. Measure the absorbance of the samples at 490 - 492 nm according to the available filters using a plate reader. The reference wavelength should be more than 600 nm.
12. Calculate the percentage cytotoxicity. (See VI-5)



Synperonic F68 (■), Triton X-100 (▲) and NP-40 (▼) were titrated in microtiter plates in culture medium as described in VII-1-1-4) to final concentrations indicated in the figure. Subsequently P815 cells were added to a final concentration of 1 x 10^4 cells/well. After incubation of the cells for 18 hrs, LDH release was determined as described in VII-1-1.

Figure 4. Measurement of the cytotoxic potential of various detergents

VII-2. Assay Procedure for Cell Mediated Cytotoxicity

1. Titrate effector cells (NK cells, LAK cells, CTLs) in the appropriate assay medium in sterile 96-well tissue culture plates by serial dilution (final volume of 100 μl/well). (Table 4, dilution 1-14).
2. After washing the target cells in assay medium, dilute cells to the optimal concentration determined in the preliminary experiment.
3. Add 100 μl of the target cell suspension to the dilutions of effector cells (effector-target cell mix) (Table 4).
4. Prepare the following controls on the plate. (See Table 3)
   1) Background control : Add 200 μl assay medium to three wells.
   2) Low control (spontaneous LDH release) : Add 100 μl of target cells to three wells containing 100 μl of assay medium.
   3) High control (maximum LDH release) : Add 100 μl of target cells to three wells containing 100 μl of Triton X-100 solution.

4) Substance control I (effector cell control, spontaneous release of LDH by the effector cells) : Add 100 μl of assay medium to three wells containing 100 μl of effector cells.

Note : The spontaneous LDH release should be determined for each effector cell concentration in the assay.

5. Incubate cells in an incubator (37℃, 5% CO_2, 90% humidity) for the time applied in assaying the test substances (2 - 24 hrs).
6. After incubation, centrifuge the microtiter plate at 250*g* for 10 min.
7. Carefully remove 100 μl/well supernatant (do not disturb the cell pellet) and transfer into corresponding wells of an optically clear 96-well flat bottom microtiter plate.
8. Add 100 μl Solution C (reaction mixture) to each well and incubate for up to 30 min at room temperature. During this incubation period, the plate should be protected from light.
9. Measure the absorbance of the samples at 490 - 492 nm according to the available filters using a plate reader. The reference wavelength should be more than 600 nm.
10. Calculate the percentage cytotoxicity. (See VI-5)

Table 4. Experimental setup to measure cell mediated cytotoxicity
(All tests should be performed in triplicate.)

	1 2 3	4 5 6	7 8 9	10 11 12
A	Background control	Target cell low control	Target cell high control	
B	Cell suspension I Dilution 1	Dilution 8	Cell suspension II Dilution 1	Dilution 8
C	Dilution 2	Dilution 9	Dilution 2	Dilution 9
D	Dilution 3	Dilution 10	Dilution 3	Dilution 10
E	Dilution 4	Dilution 11	Dilution 4	Dilution 11
F	Dilution 5	Dilution 12	Dilution 5	Dilution 12
G	Dilution 6	Dilution 13	Dilution 6	Dilution 13
H	Dilution 7	Dilution 14	Dilution 7	Dilution 14

Effector - target cell mix (columns 1–6) Effector cell control (columns 7–12)

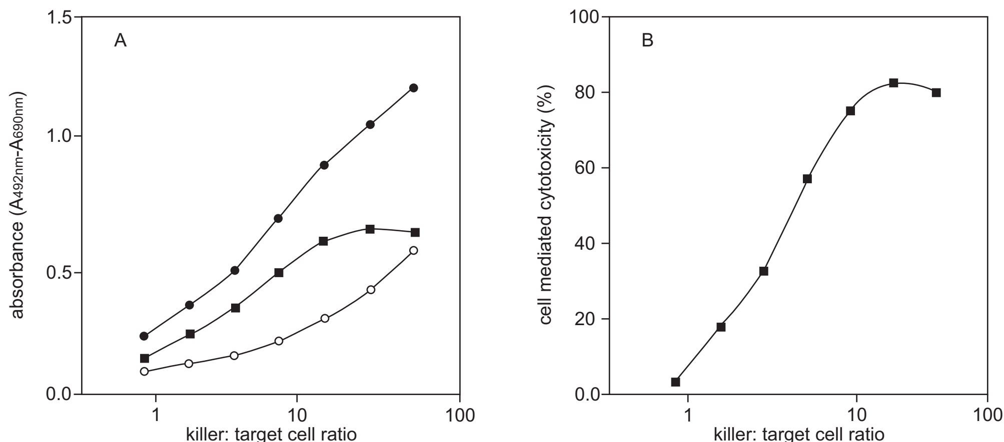


Figure 5. Measurement of the cytolytic activity of allogen-stimulated, cytotoxic T-lymphocytes (CTLs)

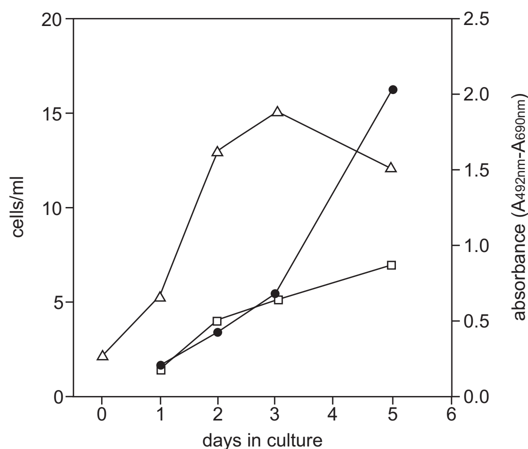
Spleen cells of C57/Bl 6 mice (H-2b) were stimulated *in vitro* with P815 cells (H-2d). Viable CTLs were purified by ficoll density gradient, washed, and then tittered in the microtiter plate as described in step VII-1. 2 - 5). 1 x 10^4 P815 cells were added to the effector cells. After 4 hours, the cells were centrifuged. Then, 100 μl of culture supernatant was removed and LDH activity was measured.

A. Absorbance values
   (○) Effector cell control
   (●) Effector-target cell mix
   (■) Effector-target cell mix (●) minus effector cell control (○)

B. Percentage cell mediated cytotoxicity, calculated as described VI-5.

VII-3. Assay of Cell Death in Eukaryotic Cell Culture

1. Collect samples (0.5 - 1 ml) at regular timepoints from 12 - 24 hrs from culture.
2. Centrifuge the sample and carefully remove culture supernatant. The cell-free supernatant can be stored at 4℃ for several days without loss of enzyme activity.
3. Titrate the culture supernatants in a microtiter plate in the appropriate culture medium by serial dilution in final volume of 100 μl/well.
4. Add 100 μl/well Solution C (reaction mixture) to each well and incubate for up to 30 min at room temperature. During this incubation period, the plate should be protected from light.
5. Measure the absorbance of the samples at 490 or 492 nm using a plate reader. The reference wavelength should be more than 600 nm.



Ag8 cells were seeded at a concentration of 2 x 10^5 cells/ml and incubated at 37℃, 5% CO_2. At day 1, 2, 3, and 5, aliquots were removed. The amount of viable (△) and dead (□) cells were determined with trypan blue exclusion. LDH activity of cell free culture supernatant (●) was determined using this kit.

Figure 6. Correlation of cell death and LDH release in cell culture

VIII. Troubleshooting

Q-1 : No color reaction

A-1 : 1) Check the cell concentration. The cell number may be too low.
2) Check test substances and/or assay medium to determine if they are contaminated with compounds that inhibit LDH activity (substance control II, see step VI-4).

Q-2 : Strong color reaction in low controls

A-2 : 1) Check cell concentration. The cell number may be too high.
2) Check test substances and/or assay medium to determine if they are contaminated with compounds that inhibit LDH activity (substance control I, see step VI-4).
3) High spontaneous release may be caused by poor condition of the cells used for assay. Check the culture conditions; some cell lines can not survive in serum free media, even for short incubation times. Increase the serum concentration to ~1 - 5%.

Q-3 : Strong color reaction but low absorbance values

A-3 : Check background values. High background values may make the absorbance values low if they are subtracted automatically. Check the assay medium for contamination with compounds with LDH activity, such as serum (substance control I, see step VI-4).

Q-4 : Strong color reaction in effector cell controls

A-4 : Poor condition of the effector cells due to inappropriate isolation method or culture conditions may be responsible. Improve cell culture and/or isolate viable effector cells by density gradient centrifugation.

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