LDH Cytotoxicity Detection Kit Manual

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1. Description

Cell death is assayed by the quantification of plasma membrane damage. Several standards methods for quantification of cellular viability were developed from the need for the sensitive, reliable and automated methods for precise determination of cell death.

Widely used standard methods are based on the uptake or exclusion of dyes such as trypan blue or eosin Y. These methods have the following disadvantages; 1) can not process large numbers of sample, 2) can not quantitate dead cells which may have damaged.
The second standard methods are based on the measurement of the amount of released radioactive isotopes or fluorescence dyes from the target cells which are prelabeled with those substances. The disadvantages of these methods are 1) need to prelabel target cells before assay, 2) prelabeled target cells release most labels spontaneously.
The third standard methods are based on the measurement of cytoplasmic enzyme activity released from damaged cells. The amount of enzyme activity correlates to the proportion of the damaged cells. Alkaline and acid phosphatase, or glutamate-oxaloacetate transaminase(GOT), or glutamate pyruvate transaminase (GPT) have been conventionally used for the methods. However, this method is not widely used because the low amount of these enzymes are present in many cells and because the kinetic assays to quantitate these enzymes are elaborate.

Lactate dehydrogenase (LDH) is a stable cytoplasmic enzyme which is present in most cells. It is released into the cell culture supernatant upon damage of the cytoplasmic membrane.

Takara's LDH Cytotoxicity Detection Kit allows simple measurement of LDH activity released from the of damaged cells into the supernatant.

2. Principle

This kit is designed to allow a precise, fast and simple colorimetric assay method to quantitate cytotoxicity/cytolysis based on the measurement of LDH activity released from damaged cells into the supernatant. This is a non-radioactive alternative assay method to the [^{3}H]-thymidine release assay and the [^{51}Cr]-release assay. The culture supernatant is collected cell-free and incubated with the reaction mixture from the kit, and the LDH activity is determined in an enzymatic test as below:
In the first step NAD^+ is reduced to $NADH/H^+$ by the LDH-catalyzed conversion of lactate to pyruvate.
In the second step, the catalyst (diaphorase) transfers H/H^+ from $NADH/H^+$ to the tetrazolium salt INT which is reduced to formazan. (See Fig.1)

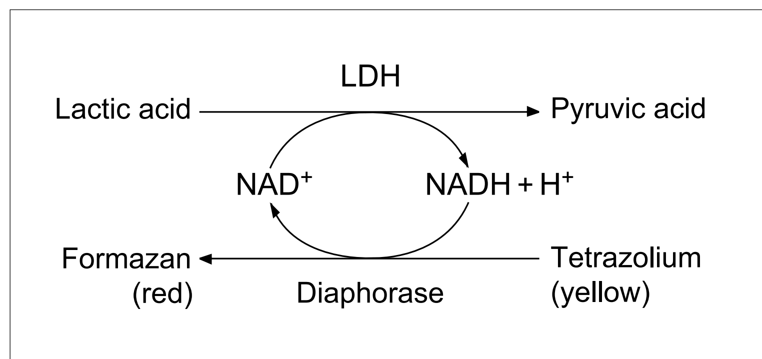

Fig. 1. Principle of measurement

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An increase in the number of dead or plasma membrane-damaged cells leads to an increase of the LDH enzyme activity in the culture supernatant. This increase of the enzyme activity in the supernatant directly correlates to the amount of formazan formed in a certain time period. That is, the amount of color formed in the assay is proportional to the number of damaged cells. The formazan dye formed is water soluble and shows the maximum absorption at about 500 nm, whereas the tetrazolium salt INT shows no significant absorption at these wave lengths. (See Fig.2)

The reaction mixture of the kit was added to RPMI1640 with 1% BSA

- In the absence of LDH : ....
- In the presence of LDH: ____

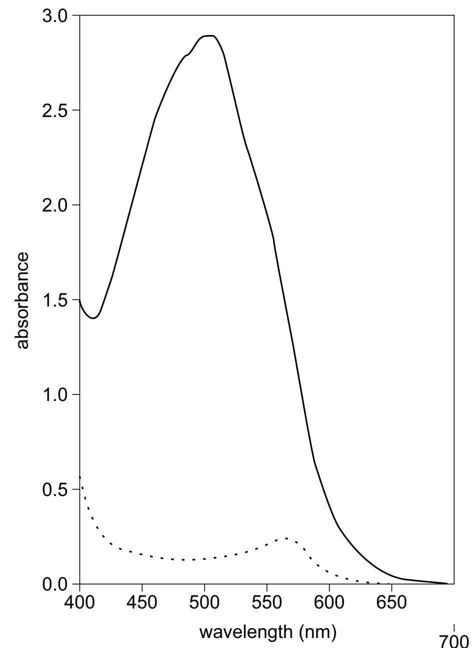

Fig.2 Absorbance spectra of the working solutions of LDH Cytotoxicity Detection Kit

This kit can be used in many various in vitro cell systems when damage to the plasma membrane occurs.

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

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3. Feature:

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

4. Kit components:

2000 tests
- Bottle 1(blue cap) x 5: Catalyst (diaphorase/ NAD^+, lyophilisate)
- Bottle 2(red cap) x 5: Dye solution (45 ml)
  (containing iodotetrazolium chloride(INT) and sodium lactate)

5. Storage:

- 20°C

• Lyophilized Catalyst can be stored for one year at -20°C, and for several weeks at 4°C. The dissolved Catalyst can be stored for several weeks at 4°C.
• The thawed Dye solution can be stored for several weeks at 4°C.

6. Preparation of solutions

6-1. Precautions

1) Assay interference
Assay may be interfered under the following conditions:
- Inherent LDH activity may be found in serum or assay substances. (See 6-2, 6-4)
- When assaying cell mediated cytotoxicity, the amount of LDH released from damaged effector cells may influence the assay results. (See 6-4, 7-2)
- Substances which interfere LDH or diaphorase enzyme activity influence assays. In this case, appropriate controls should be included in the assay. (See 6-4)

2) Sample material
Cell-free culture supernatant: Remove cells from the culture medium at around 250 x g, prior to the determination of LDH activity. Prepared culture supernatant can be stored at 4°C for a few days without loss of LDH activity.

6-2. Required equipments and reagents not supplied in the kit

Equipment

- 37°C 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 should be subtracted.)
- Microscope
- Hemacytometer
- Multichannel pipettor (100 µl)

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- Sterilized pipette tips
- 96-well microtiter plates (MTP)
  For measurement of cell mediated lysis and for the analysis of cytotoxic compounds: sterilized, cell-culture quality
  • For suspension cells - round or V-bottom
  • For adherent cells - flat bottom
  For color development in all assays - optically clear flat-bottomed

Reagents

- Assay medium
It is recommended to conduct the assay in the presence of low serum concentrations (e.g. 1%) or to replace serum by 1% bovine serum albumin (BSA) (w/v). That is because 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)
The maximum amount of releasable LDH enzyme activity can be determined by damaging cells with Triton X-100 (at final concentration : 1% Triton X-100) At this concentration, the LDH activity is not affected by Triton X-100.

- HCl stop solution (1 N)
The reaction product can be measured without adding a stop solution. However, alternatively, 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 should be calculated at unit/ml instead of relative cytotoxicity in percent or absorbance, it is recommended to prepare a standard curve using an appropriate LDH standard solution.

Please note that assay medium, lysing and stopping solutions and LDH standard solution are not included in this kit. All other reagents required to perform 2000 tests are included.

6-3. Preparation of reaction mixtures

1. Solution A: Catalyst (blue cap)
Reconstitute the lyophilisate in 1 ml of distilled water and mix thoroughly for 10 min. The reconstituted solution can be stored for several weeks at 4°C.
2. Solution B: Dye Solution (red cap)
Thawed INT dye solution is ready to use in assay without any treatment. Once thawed, the dye solution can be stored for several weeks at 4°C.
3. Solution C: Reaction mixture*
For 100 tests: Shortly before use, mix 250 μl of Solution A with 11.25 ml of Solution B.
For 400 tests: Shortly before use, mix the total volume of Solution A with the total volume of Solution B (45 ml) .

Note: The reaction mixture must be prepared immediately before use, since the reaction mixture can not be stored.

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6-4. Preparation of controls

The following three controls have to be performed in each experimental setup in order to calculate percent cytotoxicity. (See table 1)

1. Background control:
   Measure the LDH activity contained in the assay medium. The absorbance value obtained in this control has to be subtracted from all other values.
2. Low control:
   Measure the spontaneous LDH release, that is the LDH activity released from the untreated normal cells .
3. High control:
   Measure the maximum releasable LDH activity in the cells, that is the maximum LDH release induced by the addition of Triton X-100.

The following two controls are facultative:

4. Substance control I: Measure the LDH activity contained in the test substance. If cell mediated cytotoxicity is measured, this control provides information on the LDH activity released from the effector cells. (See 7-2)

5. Substance control II: Determine whether the test substance itself interferes with LDH activity. Follow the procedures as below to perform this control.

   - Add 50 µl/well test substance solution (diluted in assay medium) in triplicate in 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) (prepared at 6-3) and measure absorbance using an ELISA reader. Compare the measured absorbance values with those absorbance values obtained with the control sample which was prepared by mixing 50 µl/well LDH solution (0.05 units/ml), 50 µl/well assay medium and 100 µl/ml reaction mixture.

Contents of the well	Background control**	Low control**	High control**	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*	-	-	100 µl	-	-	-
Test substance of effector cells	-	-	-	100 µl	50 µl	100 µl
LDH standard	-	-	-	-	50 µl	-

Note: * Triton X-100 solution: prepared 2% in the assay medium
** The background, low and high controls have to be determined in each experimental setup.

Table 1. Overview of the controls

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6-5. Calculation of cytotoxicity

• Calculation of percentage cytotoxicity
The percentage cytotoxicity is determined by calculating the average absorbance values of the triplicates and subtracting from each of these the absorbance value obtained in the background control. The resulting values are substituted into the following equation:

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

• Calculation of percentage cell mediated cytotoxicity
The percentage cell mediated cytotoxicity is determined by calculating the average absorbance of the triplicates and subtracting the background. These values are substituted into 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]

6-6. Preliminary experiment

(Determination of the optimal cell concentration of the target cell)

Amounts of LDH differs depending on cell types. Therefore, the optimum cell concentration for a specific cell type should be determined in a preliminary experiment. In general, this cell concentration is at the point where the difference between the low and high control is at a maximum. This optimal concentration should be used for the subsequent assay. With 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 the entire 96-well tissue culture plate with 100 μl/well assay medium.
2. After washing cells with assay medium, adjust cell suspension 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. (See Table 2.) Titrate 100 μl/well 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 by 14 times until Dilution 14 is prepared.
4. Prepare the following controls on the plate.
   1) Determine the background control: Fill 200 μl assay medium into triplicate wells. (A1-A3, Table 2)
   2) Determine the low control (=spontaneous LDH release): Add 100 μl/well assay medium to triplicate wells containing 100 μl/well cells. (1-6 of B-H, Table 2)
   3) Determine the high control (=maximum LDH release): Add 100 μl/well Triton X-100 solution to triplicate wells containing 100 μl/well cells. (B-H 7-12, Table 2)
5. Incubate the cells in an incubator (37°C, 5% CO_2, 90% humidity) for the time applied in assaying the test substances.
6. Centrifuge the microtiter plate at 250 x g for 10 min.
7. Remove 100 μl/well supernatant carefully (do not disturb the cell pellet), transfer into corresponding wells of an optically clear 96-well flat bottom microtiter plate (MTP).

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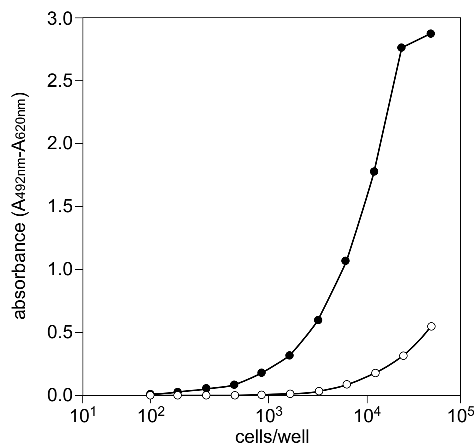
8. To determine the LDH activity in these supernatants, add 100 μl reaction mixture to each well and incubate at room temperature for 30 min. During this incubation period, the MTP should be protected from light.
9) Measure the absorbance of the samples at 490 or 492 nm according to the filters available using an ELISA reader. 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 horizontal 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 at about 1 x 10^4 cells/well.

Fig.3 Determination of the optimal target cell concentration for K562 cells

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7. Protocol

Quick reference protocols

Step	Procedure	Volume/well	Time/Temperature
1.	Incubate target cells with test substance or cytotoxic effector cells.	200 µl	4-24 hrs/37°C
2.	Centrifuge the MTP containing the cells.		10 min, 250 x g at RT
3.	Transfer cell free culture supernatant to clear, flat bottom MTP.	100 µl	
4.	Add Solution C (reaction mixture) and incubate to react cells with LDH.	100 µl	approx. 10-30 min. at RT, protected from light.
5.	If the reaction is to be stopped, add 1 N HCl to each well.	50 µl	
6.	Measure absorbance at about 490 nm. (reference wave length 690 nm)		

MTP: microtiter plate, RT: room temperature

7-1. 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 (6-6).
3. Add 100 µl/well cell suspension with the optimal concentration into the dilutions of the test substances.
4. Prepare the following controls on the plate. (See Table 3)
   1) Determine the background control: Fill 200 µl assay medium into triplicate wells.
   2) Determine the low control (=spontaneous LDH release): Add 100 µl/well cell suspension to triplicate wells containing 100 µl/well assay medium.
   3) Determine the high control (=maximum LDH release): Add 100 µl/well suspension to triplicate wells containing 100 µl/well Triton X-100 solution.
   4) Determine the substance control I: Add 100 µl/well test substance (with maximum concentration used in the experiment) to triplicate wells containing 100 µl/well assay medium.
5. Incubate the cells in an incubator (at 37°C, 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 x 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 (MTP).
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 MTP should be protected from light.
9. Measure the absorbance of the samples at 490 - 492 nm according to the filter available using an ELISA reader. The reference wavelength should be more than 600 nm.
10. Calculate the percentage cytotoxicity. (See 6-5)

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

	1 2 3	4 5 6	7 8 9	10 11 12
A	Background control	Test substance 1 Substance control I (Dilution 1)	Test substance 2 Substance control I (Dilution 1)	
B	Test substance 1 Dilution 1	Dilution 8	Test substance 2 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	Low control	Dilution 7	High control

Two-fold dilution series of test substance 1 + cell suspension

Two-fold dilution series of test substance 2 + cell suspension

2. Assay procedure for adherent cells

1. After washing the cells in assay medium, dilute to the concentration determined in the preliminary experiment. Add 100 µl/well cell suspension per well in 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°C, 5%, CO_2, 90% humidity) to allow the cells to adhere tightly.
3. Immediately before use, titrate test substances (mediators, cytolytic or cytotoxic agents) in the appropriate assay medium in a separate MTP by serial dilutions (final volume of 200 µl/ml).
4. Remove the assay medium from the adherent cells (to remove LDH activity released from the cells during the overnight incubation step). Add 100 µl fresh assay medium to each well.
5. Transfer 100 µl of the test substance dilutions into corresponding wells containing adherent cells.
6. Prepare the following controls on the plate. (See Table 3)
   Determine the background control: Fill 200 µl assay medium into triplicate wells.
   1) Determine the low control (=spontaneous LDH release): Add 100 µl/well assay medium into triplicate wells containing 100 µl/well cells.
   2) Determine the high control (=maximum LDH release): Add 100 µl/well Triton X-100 solution to triplicate wells containing 100 µl/well cells.
   3) Determine the substance control I: Add 100 µl/well test substance (maximum concentration used in the experiment) to triplicate wells containing 100 µl/well assay medium.

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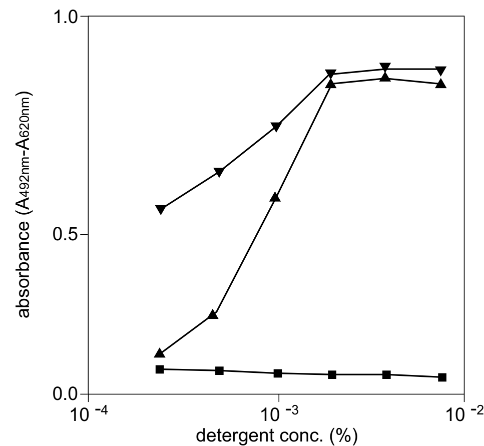
7. Incubate the cells in an incubator (at 37°C, 5% CO_2, 90% humidity) for the time applied in assaying the test substances (Incubation time differs in the range of 2 - 24 hrs dependent on the experimental setup).
8. After incubation, centrifuge the microtiter plate at 250 x g for 10 min.
9. Carefully 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 (MTP).
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 contained in the supernatant. During this incubation period, the MTP has to be protected from light.
11. Measure the absorbance of the samples at 490 - 492 nm according to the filter available using an ELISA reader. The reference wavelength should be more than 600 nm.
12. Calculate the percentage cytotoxicity. (See 6-5)

Fig. 4 Measurement of the cytotoxic potential of various detergents

Symperonic FG8(■), Triton X-100(▲) and Nonident P-40(▼) were titrated in microtiter plates in culture medium as described in 7-1.1 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 7-1.1.



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7-2. 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 dilutions. (final volume of 100 µl/ml). (See Table 4, Dilution 1-14)
2. After washing the target cells in assay medium, dilute cells to the concentration determined in the preliminary experiment.
3. Add 100 µl/well target cell suspension to the dilutions of effector cells (=effector-target cell mix). (See Table 4).
4. Prepare the following controls on the plate. (See Table 3)
   1) Determine the background control: Fill 200 µl assay medium into triplicate wells.
   2) Determine the low control (=spontaneous LDH release): Add 100 µl/well target cells into triplicate wells containing 100 µl/well assay medium.
   3) Determine the high control (maximum LDH release): Add 100 µl/well target cells into triplicate wells containing 100 µl/well Triton X-100 solution.
   4) Determine the substance control I (=effector cell control = spontaneous release of LDH by the effector cells): Add 100 µl/well assay medium to triplicate wells containing 100 µl/well effector cells.
   Note: The spontaneous LDH release should be determined for each effector cell concentration applied in the assay.
5. Incubate cells in an incubator (at 37°C, 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 x 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 (MTP).
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 MTP should be protected from light.
9. Measure the absorbance of the samples at 490 - 492 nm according to the filter available using an ELISA reader. The reference wavelength should be more than 600 nm.
10. Calculate the percentage cytotoxicity. (See 6-5)

	1 2 3	4 5 6	7 8 9	10 11 12
A	Background control	Targe cell low control	Target cell high control	
B	Dilution 1	Dilution 8	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		Effector cell control	

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

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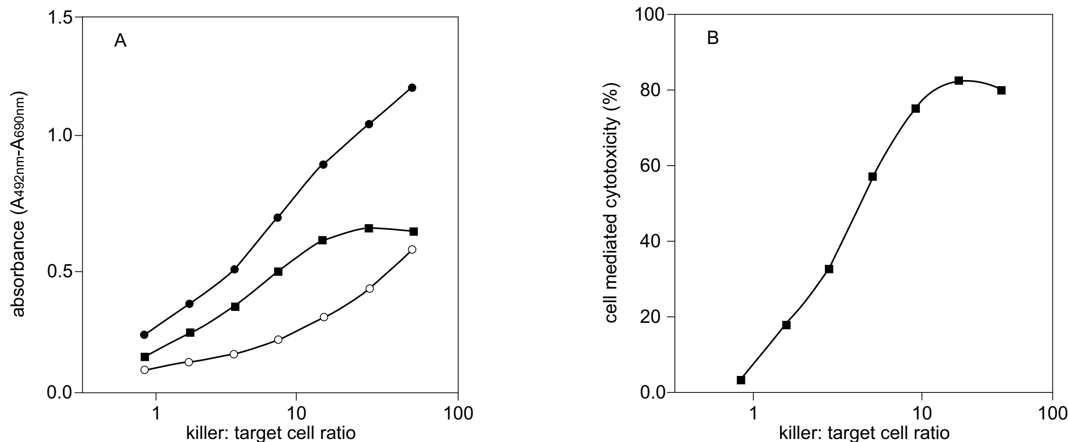
Fig.5 Measurement of the cytolytic activity of allogen-stimulated, cytotoxic T-lymphocytes (CTLs)



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 7-1.2 5). 1 x 10^4 P815 target cells/well were added to the effector cells. After incubation of 4 hours, the cells were centrifuged. Then 100 µl of culture supernatant were 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 6-5.

7-3. Assay of cell death in eukaryotic cell fermentation

1. Collect samples (0.5 - 1 ml) at regular intervals of 12 - 24 hrs from cell culture.
2. Centrifuge the sample and carefully remove culture supernatant. The cell-free supernatant can be stored at 4°C without loss of enzyme activity for a few days.
3. Titrate the culture supernatants to the microtiter plate (MTP) in the appropriate culture medium by serial dilutions to obtain a 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 MTP has to be protected from light.
5. Measure the absorbance of the samples at 490 or 492 nm using microtiter plate (ELISA) reader. The reference wavelength should be more than 600 nm.

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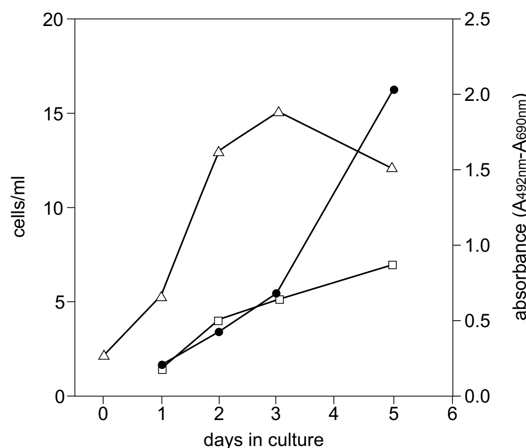
Fig.6 Correlation of cell death and LDH release in cell culture

Ag8 cells were seeded at a concentration of 2 x 10^8 cells/ml and incubated at 37°C, 5% CO_2. At day 1, 2, 3 and 5, culture aliquotes were removed for samples. 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.



8. Trouble shooting

Q-1: No color reaction at all.
A-1: 1) Check cell concentration. May be too low.
2) Check test substances and/or assay medium if compounds inhibiting LDH activity may be contaminated. (Substance control II, See 6-4)

Q-2: Strong color reaction also in low controls
A-2: 1) Check cell concentration: May be too high.
2) Check test substances and/or assay medium if compounds inhibiting LDH activity may be contaminated. (Substance control I, See 6-4)
3) High spontaneous release may be caused by bad condition of the cells used in the assay. Check culture conditions: some cell lines can not survive in serum free media, even in short incubation times. Increase the serum concentration to about 1-5%.

Q-3: Strong color reaction but low absorbance values
A-3: Check background values. High background values may make the absorbance values too low if they are subtracted automatically. Check assay medium if compounds with LDH activity may be contaminated. (Sera, substance control I, See 6-4.)

Q-4: Strong color reaction at effector cells controls
A-4: Bad conditions of the effector cells due to inappropriate isolation method or culture conditions may be responsible. Improve cell culture. Separate viable effector cells from dead ones by density gradient centrifugation.

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LDH Cytotoxicity Detection Kit Manual

9. References

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Note: This product is for research use only.
Not for use in diagnostic procedures for clinical purposes. For *in vitro* use only.

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