TECHNICAL BULLETIN

CytoTox 96® Non-Radioactive Cytotoxicity Assay

Instructions for Use of Product
G1780

Revised 7/16
TB163

CytoTox 96® Non-Radioactive Cytotoxicity Assay

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1. Description

The CytoTox 96® Non-Radioactive Cytotoxicity Assay is a colorimetric alternative to ^{51}Cr release cytotoxicity assays. The CytoTox 96® Assay quantitatively measures lactate dehydrogenase (LDH), a stable cytosolic enzyme that is released upon cell lysis, in much the same way as ^{51}Cr is released in radioactive assays. The half-life of LDH that has been released from cells into the surrounding medium is approximately 9 hours. Released LDH in culture supernatants is measured with a 30-minute coupled enzymatic assay, which results in the conversion of a tetrazolium salt (iodonitrotetrazolium violet; INT) into a red formazan product. The amount of color formed is proportional to the number of lysed cells. Visible wavelength absorbance data are collected using a standard 96-well plate reader. Methods for measuring LDH using tetrazolium salts in conjunction with diaphorase or alternate electron acceptors have been used for many years (1). Variations on this technology have been reported for measuring natural cytotoxicity and are identical (within experimental error) to values determined in parallel ^{51}Cr release assays (2,3).

The general chemical reactions of the CytoTox 96® Assay are illustrated in Figure 1.

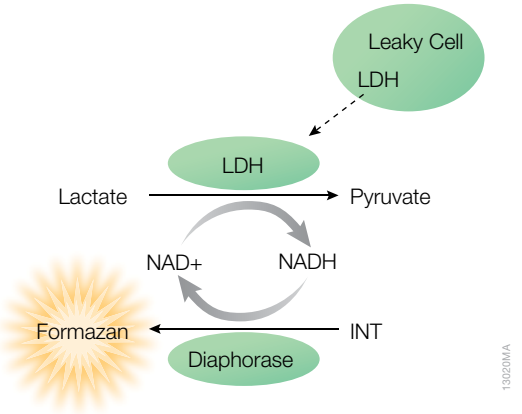


Figure 1. Release of LDH from damaged cells is measured by supplying lactate, NAD+ and INT as substrates in the presence of diaphorase. Generation of a red formazan product is proportional to the amount of LDH released and therefore the number of lysed cells.

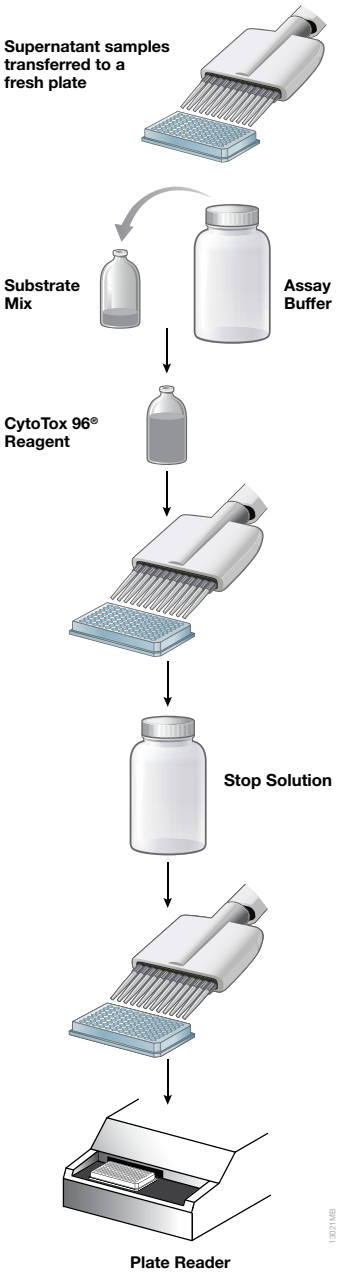
Applications of the CytoTox 96® Assay

- cytotoxicity mediated by chemicals or other agents (4–7)
- total cell number (8)
- cell-mediated cytotoxicity (9)

Advantages of the CytoTox 96® Assay

- eliminates labeling of cells in cell-mediated cytotoxicity experiments
- eliminates paperwork and safety issues of radioactivity
- allows use of standard plate reader
- can reveal early, low-level cytotoxicity

Figure 2. The CytoTox 96® Non-Radioactive Cytotoxicity Assay protocol. Following experimental treatment, supernatant samples are transferred to a 96 or 384-well plate and an equal volume of CytoTox 96® Reagent is added to each well and incubated for 30 minutes. Stop Solution is added, and the absorbance signal is measured at 490nm in a plate reader.



2. Product Components and Storage Conditions

PRODUCT	SIZE	CAT.#
CytoTox 96® Non-Radioactive Cytotoxicity Assay	**1,000 assays**	**G1780**

G1780 contains sufficient Substrate Mix, Assay Buffer and Stop Solution for 1,000 cell-mediated cytotoxicity assays in 96-well plate format. Includes:

- 5 vials Substrate Mix
- 60ml Assay Buffer
- 25µl LDH Positive Control
- 5ml Lysis Solution (10X)
- 65ml Stop Solution

Available Separately

PRODUCT	SIZE	CAT.#
Lysis Solution	**5ml**	**G1821**

Storage Conditions: Store Substrate Mix and Assay Buffer frozen at –20°C protected from light. CytoTox 96® Reagent (Assay Buffer combined with Substrate Mix) may be stored for 6–8 weeks at –20°C protected from light without loss of activity. Store LDH Positive Control, Lysis Solution (10X) and Stop Solution at 4°C.

Note: Upon storage, a precipitate might form in the Assay Buffer. This precipitate does not affect assay performance. The precipitate may be removed by centrifugation at 300 × *g* for 5 minutes. Use 12ml of the supernatant to reconstitute the Substrate Mix.

3. Reagent Preparation

Thaw Assay Buffer, remove 12ml and promptly store the unused portion at –20°C. Warm 12ml of Assay Buffer to room temperature; keep protected from light. Add 12ml of room-temperature Assay Buffer to a bottle of Substrate Mix to form the CytoTox 96® Reagent. Invert and shake gently to dissolve the substrate.

Notes:

1. A 37°C water bath may be used to thaw the Assay Buffer, but return the Assay Buffer to –20°C storage protected from light as soon as it is thawed and used.
2. Upon storage, a precipitate might form in the Assay Buffer. This precipitate does not affect assay performance. The precipitate may be removed by centrifugation at 300 × *g* for 5 minutes. Use 12ml of the supernatant to reconstitute the Substrate Mix.
3. One bottle of CytoTox 96® Reagent is enough for two 96-well plates (200 assays) when using 50µl samples.
4. Once resuspended, protect the CytoTox 96® Reagent from strong direct light and use immediately. Store any unused Reagent at –20°C.

4. Cytotoxicity Assay

The CytoTox 96® Assay can be used to measure cell death following treatment with a cytotoxic drug or compound (10).

Materials to Be Supplied By the User

- 96- or 384-well culture plates compatible with a standard plate reader
- multichannel pipettor
- reservoirs to hold CytoTox 96® Reagent and Stop Solution
- plate reader capable of recording absorbance 490 or 492nm
- plate shaker

4.A. Recommended Controls

Perform each of these controls on each plate being assayed.

No-Cell Control: Set up triplicate wells without cells to serve as the negative control to determine culture medium background.

Vehicle-Only Cells Control: Set up triplicate wells with untreated cells to serve as a vehicle control. Add the same solvent used to deliver the test compounds to the vehicle control wells.

Maximum LDH Release Control (Optional): Set up triplicate wells to determine the Maximum LDH Release Control. Add 10µl of 10X Lysis Solution per 100µl of Vehicle-Only Cells Control 45 minutes before adding CytoTox 96® Reagent. **Note:** This control is only required if calculating % cytotoxicity (see Section 4.C).

4.B. Cytotoxicity Assay Protocol

1. Set up 96-well assay plates containing cells in culture medium. Be sure to prepare wells for the recommended controls (Section 4.A).

   Note: For 384-well plates, the recommended culture volume is 25–30µl per well.

2. Add test compounds and vehicle controls to appropriate wells so the final volume is 100–150µl in each well (25–30µl for a 384-well plate).

3. Incubate cells at 37°C for the desired test exposure period.

   Note: LDH has a half-life of approximately 9 hours when released into cell culture media. The test exposure period will have to be optimized.

4. **Optional:** If Lysis Solution is used to generate a Maximum LDH Release Control, add 10µl of 10X Lysis Solution (per 100µl original volume) to the positive control wells 45 minutes before adding CytoTox 96® Reagent.

5. Transfer 50µl aliquots from all test and control wells to a fresh 96-well flat clear bottom plate. For 384-well format, transfer 12.5µl aliquots.

4.B. Cytotoxicity Assay Protocol (continued)

6. Add 50µl of the CytoTox 96® Reagent to each sample aliquot. For 384-well format, add 12.5µl to each sample aliquot. Cover the plate with foil or an opaque box to protect it from light and incubate for 30 minutes at room temperature.
7. Add 50µl of Stop Solution to each well of the 96-well plate. For 384-well plates, add 12.5µl of Stop Solution.
8. Pop any large bubbles using a syringe needle, and record the absorbance at 490nm or 492nm within 1 hour after adding the Stop Solution.

4.C. Calculation of Results

1. Subtract the average values of the culture medium background from all values of experimental wells.
2. Use the corrected values in the following formula to compute percent cytotoxicity:

$$\text{Percent cytotoxicity} = 100 \times \frac{\text{Experimental LDH Release } (OD_{490})}{\text{Maximum LDH Release } (OD_{490})}$$

5. Cell-Mediated Cytotoxicity Assay

Materials to Be Supplied by the User

(Solution compositions are provided in Section 10.A.)

- 96-well flat-bottom culture plates compatible with a standard plate reader
- round- or V-bottom 96-well tissue culture plates
- multichannel pipettor
- plate reader capable of recording absorbance 490 or 492nm
- **Optional:** PBS + 1% BSA (bovine serum albumin)

5.A. Optimization of Target Cell Number

Because various target cell types (YAC-1, K562, Daudi, etc.) contain different amounts of LDH, we recommend a preliminary experiment to determine the optimum number of target cells to use with the CytoTox 96® Assay and to ensure an adequate signal-to-noise ratio. The LDH Positive Control supplied may be used to verify that the LDH assay is functioning properly.

1. Prepare target cell dilutions (e.g., 0, 5,000, 10,000, 20,000/100µl) in cell culture medium. Use the same medium and final volume that will be used for cytotoxicity assays.
2. Add 100µl of cells per well to a round- or V-bottom 96-well plate.
3. Lyse cells by adding 10µl of Lysis Solution per 100µl of medium (or lyse the cells by freezing and thawing).
4. Centrifuge plate at 250 × *g* for 4 minutes.
5. Transfer 50µl of supernatant from all wells to a fresh 96-well flat-bottom (enzymatic assay) plate.
6. Add 50µl of CytoTox 96® Reagent to each well of the enzymatic assay plate.
7. Cover the plate with foil or an opaque box to protect it from light and incubate for 30 minutes at room temperature.
8. Add 50µl of Stop Solution to each well.
9. Pop any large bubbles using a syringe needle, and record the absorbance at 490nm or 492 nm within 1 hour after adding Stop Solution.

   Note: For cell-mediated cytotoxicity assays that co-culture 100µl/well of target cells with 100µl/well effector cells, target cell sensitivity can be increased by co-culturing the same number of cells in 50µl/well volumes. By doing this, the concentration of released cellular LDH is increased.

5.B. Protocol

Assay Plate Setup

1. Prepare serial dilutions of each effector cell type in triplicate or quadruplicate sets of wells in a round- or V-bottom 96-well tissue culture plate. Use the same medium and final volume that will be used for cytotoxicity assays. For example, if you normally co-culture 50µl/well of target cells with 50µl/well of effector cells, prepare serial dilutions in 100µl/well.
2. Prepare a triplicate or quadruplicate set of wells for the Culture Medium Background without cells.
3. **Optional:** If you wish to perform an LDH positive control, gently mix the LDH Positive Control by vortexing, and then dilute 2µl of this solution into 10ml of PBS + 1% BSA (1:5,000 dilution). Prepare this stock solution fresh for each use. Use a volume equivalent to that used for the wells containing cells. We recommend triplicate or quadruplicate wells.
4. Set up the 96-well assay plate using the following guidelines. Perform each experimental and control reaction in triplicate or quadruplicate. A suggested plate setup is shown in Figure 3.

   Effector Cell Spontaneous LDH Release: Add effector cells at each concentration used in the experimental setup to a triplicate or quadruplicate set of wells containing medium to obtain the effector cell spontaneous release. The final volume must be the same as that in the experimental wells (use medium alone with no cells to bring up the volume).

   Experimental Wells: Add a constant number of target cells (determined in Section 5.A) to all experimental wells of a V- or round-bottom 96-well culture plate. Add various numbers of effector cells to triplicate or quadruplicate sets of wells to test several effector:target cell ratios. The final combined volume per well should be a minimum of 100µl.

   Target Cell Spontaneous LDH Release: Add target cells (concentration determined in Section 5.A) to a triplicate or quadruplicate set of wells containing culture medium. The final volume must be the same as that in the experimental wells containing both target and effector cells (use culture medium to adjust volume).

   Target Cell Maximum LDH Release: Add target cells (concentration determined in Section 5.A) to a triplicate or quadruplicate set of wells containing culture medium. The final volume must be the same as that in the experimental wells. Add 10µl of the Lysis Solution (10X) per 100µl of culture medium. This will result in a concentration of approximately 0.8% Triton® X-100, which should yield complete lysis of target cells. Incubate target cells in the presence of Lysis Solution for 45 minutes prior to harvesting the supernatants.

   Volume Correction Control: Add 10µl of Lysis Solution (10X) to a triplicate or quadruplicate set of wells containing 100µl of culture medium (without cells). This control is recommended to correct for the volume increase caused by the addition of Lysis Solution (10X). This volume change affects the concentration of phenol red and serum, which contribute to the absorbance readings.

   Culture Medium Background Control: Add 100µl of culture medium to a triplicate or quadruplicate set of wells. This control is required to correct for contributions caused by phenol red and LDH activity that may be present in serum-containing culture medium.

LDH Positive Control (optional): We have included a positive control (bovine heart LDH) to verify performance of other system components. If you wish to perform an LDH positive control, gently mix the LDH Positive Control by vortexing, and then dilute 2µl of this solution into 10ml of PBS + 1% BSA (1:5,000 dilution). Prepare this stock solution fresh for each use. The final volume must be the same as that in the experimental wells. A 1:5,000 dilution of the LDH Positive Control will give approximately the same level of enzyme found in 13,500 lysed L929 fibroblast cells. We recommend triplicate or quadruplicate wells.

5. Centrifuge the assay plate at 250 × *g* for 4 minutes to ensure effector and target cell contact.

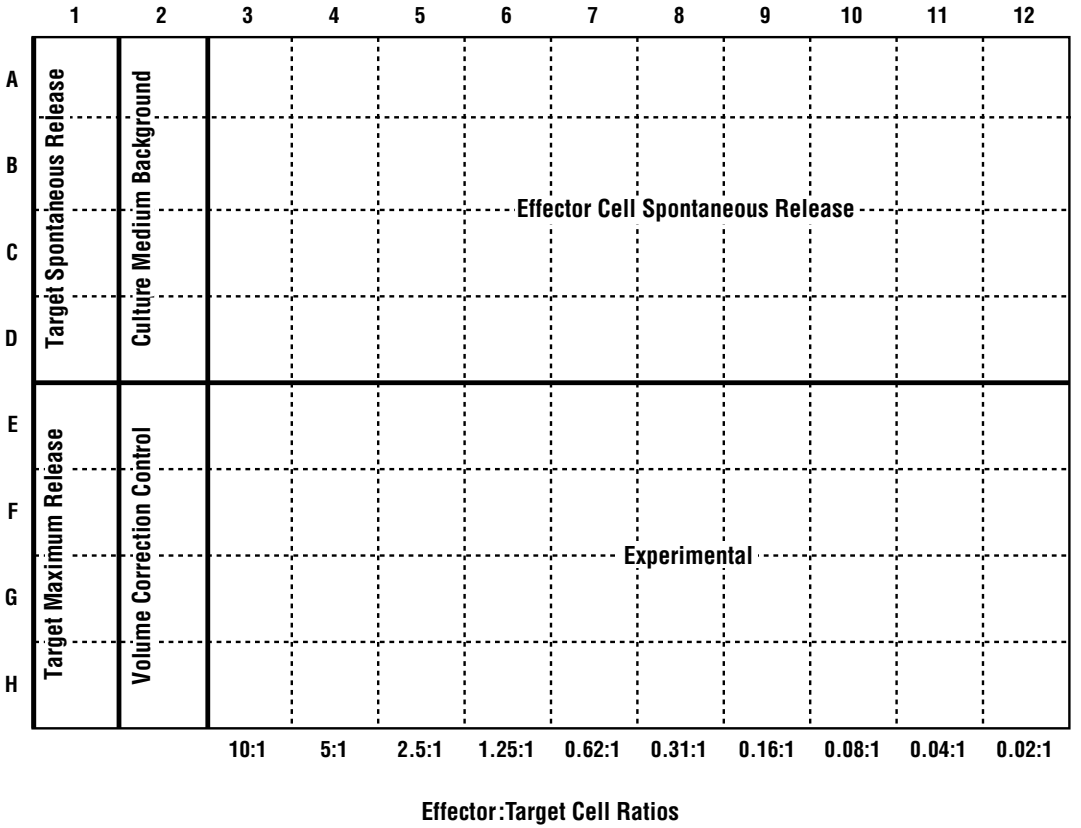


Figure 3. Representative CytoTox 96® Non-Radioactive Cytotoxicity Assay plate setup. The drawing is a representation of a 96-well plate containing the experimental and control wells necessary for the cell-mediated cytotoxicity assay. Perform each experimental and control reaction in triplicate or quadruplicate.

5.B. Protocol (continued)

Cell Culture and Supernatant Harvest

6. Incubate the cytotoxicity assay plate for 4 hours in a humidified chamber at 37°C, 5% CO_2. A minimum 4-hour incubation is needed for sufficient contact between target and effector cells.
7. Forty-five minutes prior to harvesting supernatants, add 10µl of Lysis Solution (10X) for every 100µl of target cells to the wells containing the Target Cell Maximum LDH Release Control.

   Note: If the target cells are not completely lysed (as determined by microscopy), add another 5µl of Lysis Solution (10X).
8. After the 4-hour incubation, centrifuge the plate at 250 × *g* for 4 minutes.

LDH Measurement

9. Transfer 50µl aliquots from all wells using a multichannel pipettor to a fresh 96-well flat-bottom (enzymatic assay) plate.
10. Add 50µl of CytoTox 96® Reagent to each well of the enzymatic assay plate containing samples transferred from the cytotoxicity assay plate in Section 5.B, Step 9. Cover the plate with foil or an opaque box to protect it from light and incubate for 30 minutes at room temperature.

    Note: Store unused portions of the CytoTox 96® Reagent tightly capped at –20°C for ≤6–8 weeks.
11. Add 50µl of Stop Solution to each well.
12. Pop any large bubbles using a syringe needle, and record the absorbance at 490nm or 492nm within 1 hour after adding Stop Solution.

5.C. Calculation of Results

1. Subtract the average absorbance value for the Culture Medium Background from all absorbance values for Experimental, Target Cell Spontaneous LDH Release and Effector Cell Spontaneous LDH Release.
2. Subtract the average absorbance values for the Volume Correction Control from the absorbance values obtained for the Target Cell Maximum LDH Release Control.
3. Use the corrected values obtained in Steps 1 and 2 in the following formula to calculate percent cytotoxicity for each effector:target cell ratio.

$$\% \text{ Cytotoxicity} = \frac{\text{Experimental} - \text{Effector Spontaneous} - \text{Target Spontaneous}}{\text{Target Maximum} - \text{Target Spontaneous}} \times 100$$

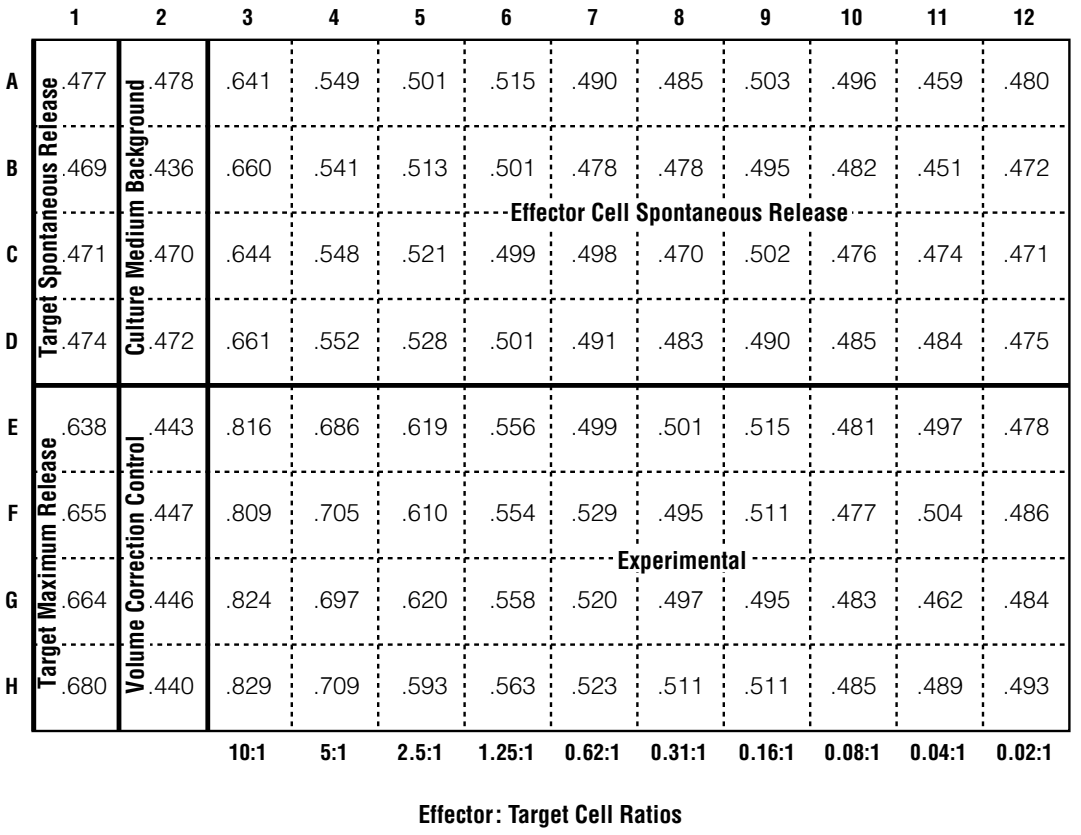


Figure 4. Representative data from a cell-mediated cytotoxicity assay. The assay conditions used to generate these data are described in Section 5.B.

5.C. Calculation of Results (continued)

Sample Calculations

The following sample calculations are based on the data shown in Figure 4, obtained using the CytoTox 96® Assay and the following experimental conditions.

Effector Cells: NK/LAK cells generated from male C3H/HeJ mice. Nylon wool-nonadherent spleen cells were cultured with rhIL-2 (500ng/ml) for 5 days prior to use in the cytotoxicity assay.

Target Cells: YAC-1 cells maintained as an upright suspension line prior to use in the assay.

Culture Medium in Assay: RPMI 1640 (containing phenol red) + 15mM HEPES + 5% FBS.

Plate: 96-well round-bottom plate.

Target Cell Plating: 10,000 cells/well in 50µl medium.

Effector Cell Plating: Ratios of 10:1 to 0.02:1 in 50µl medium.

Incubation: Four hours at 37°C, 5% CO_2.

1. Experimental, 10:1 cell ratio (avg.) − Culture Medium Background (avg.) = 0.819 − 0.464 = 0.355

   Target Spontaneous (avg.) − Culture Medium Background (avg.) = 0.472 − 0.464 = 0.008

   Effector Spontaneous (avg.) − Culture Medium Background (avg.) = 0.651 − 0.464 = 0.187

2. Target Maximum (avg.) − Volume Correction Control (avg.) = 0.659 − 0.444 = 0.215

3. $$\% \text{ Cytotoxicity} = \frac{\text{Experimental} - \text{Effector Spontaneous} - \text{Target Spontaneous}}{\text{Target Maximum} - \text{Target Spontaneous}} \times 100$$

   $$\textbf{\% Cytotoxicity} = \frac{0.355 - 0.187 - 0.008}{0.215 - 0.008} \times 100$$

   = **77.3%** for the 10:1 effector:target cell ratio

6. Total Cell Number Assay

The CytoTox 96® Assay indirectly measures lactate dehydrogenase activity present in the cytoplasm of intact cells. Cell quantitation, therefore, can occur only if the cells are lysed to release the LDH present in the cell. Some detergents (SDS and cetrimide) inhibit the generation of the final red formazan product. However, the Lysis Solution included with the CytoTox 96® Assay can be used for cell lysis and does not interfere with the assay when used as recommended. To perform the total cell number assay, additional Lysis Solution is required. Lysis Solution (Cat.# G1821) is available separately or can be prepared using Triton® X-100 (Section 10.A).

Cell samples of interest are lysed by adding 15µl of Lysis 10X Solution [9% (v/v) Triton® X-100 in water] per 100µl of culture medium, followed by incubation at 37°C for 45–60 minutes. Sample supernatants (50µl) are then transferred to a fresh 96-well enzymatic assay plate. CytoTox 96® Reagent (50µl) is added to each supernatant sample, and the enzymatic reaction is allowed to proceed for 30 minutes at room temperature, protected from light. The enzymatic assay is then stopped by adding 50µl/well of Stop Solution. Absorbance can be read at 490nm using a plate reader. The number of cells present will be directly proportional to the absorbance values, which represent LDH activity. Resulting data can be plotted with absorbance at 490nm values along the Y axis and cell number along the X axis.

Materials to Be Supplied By the User

- 96-well flat-bottom culture plates compatible with a standard plate reader
- plate reader capable of recording absorbance at 490 or 492nm

Protocol

1. Add 100µl of cells in cell culture medium to experimental wells of a 96-well plate.
2. Add 100µl of culture medium to separate wells for background control.
3. Add 15µl of Lysis Solution (10X) to **all** wells. Incubate for 45–60 minutes at 37°C.
4. Transfer 50µl of supernatant to each well of a fresh 96-well flat-bottom (enzymatic assay) plate.
5. **Optional:** If you wish to perform an LDH positive control, gently mix the LDH Positive Control by vortexing, and then dilute 2µl of this solution into 10ml of PBS + 1% BSA (1:5,000 dilution). Prepare this stock solution fresh for each use. Use a volume equivalent to that used for the wells containing cells. We recommend triplicate or quadruplicate wells.
6. Add 50µl of CytoTox 96® Reagent to each well of the enzymatic assay plate.
7. Cover plate with foil or an opaque box to protect it from light, and incubate for 30 minutes at room temperature.
8. Add 50µl of Stop Solution to each well.
9. Pop any large bubbles using a syringe needle, and record the absorbance at 490nm or 492nm within 1 hour after adding Stop Solution.

7. General Considerations

7.A. Background Absorbance Corrections

Many tissue culture media contain LDH from animal sera and phenol red, which can both contribute to background absorbance using the CytoTox 96® Assay. Background absorbance from these factors can be corrected for by including a culture medium background control. The absorbance value determined from this control is used to normalize the absorbance values obtained from the other samples (see Section 5.B). Background absorbance from phenol red also may be eliminated by using a phenol red-free medium.

The quantity of LDH in animal sera will vary depending on several parameters, including the species and the health or treatment of the animal prior to collecting serum. Human AB serum is relatively low in LDH activity, whereas calf serum is relatively high. The concentration of serum can be decreased to reduce the amount of LDH contributing to background absorbance (3). In general, decreasing the serum concentration to 5% will significantly reduce background without affecting cell viability. The use of 1% BSA in place of serum is not recommended for cell-mediated cytotoxicity assays.

7.B. CytoTox 96® Assay Controls

The five controls listed below must be performed with CytoTox 96® cell-mediated cytotoxicity assays. Controls #2 and #3 are identical to those in a standard ^{51}Cr release assay (target cell spontaneous release and target cell maximum release). The three additional controls account for LDH activity contributed from other sources.

1. **Effector Cell Spontaneous LDH Release:** Corrects for spontaneous release of LDH from effector cells.
2. **Target Cell Spontaneous LDH Release:** Corrects for spontaneous release of LDH from target cells.
3. **Target Cell Maximum LDH Release:** Required in calculations to determine 100% release of LDH.
4. **Volume Correction Control:** Corrects for volume change caused by addition of Lysis Solution (10X).

   Note: The Volume Correction Control may be eliminated if freeze-thaw lysis is substituted for addition of Lysis Solution (10X) to obtain Target Cell Maximum LDH Release.
5. **Culture Medium Background:** Corrects for LDH activity contributed by serum in culture medium and the varying amounts of phenol red in the culture medium.

8. Troubleshooting

For questions not addressed here, please contact your local Promega Branch Office or Distributor. Contact information available at: **www.promega.com**. E-mail: **techserv@promega.com**

Symptoms	Causes and Comments
High background absorbance	Endogenous LDH in animal sera in culture medium. This background absorbance normally is accounted for by the Culture Medium Background. To reduce background absorbance, change the source of serum or reduce the serum concentration. LDH activity in sera vary, with human AB serum, horse serum, fetal bovine serum and calf serum containing increasing levels of LDH activity. In general, decreasing serum concentration to 5% will significantly reduce background without affecting cell viability. Using 1% BSA in place of serum is not recommended for cell-mediated cytotoxicity assays.
	Phenol red in culture medium. This background absorbance normally is accounted for by the Culture Medium Background. A phenol red-free medium may be used, if desired.
High absorbance for Effector Cell or Target Cell Spontaneous LDH Release Controls	"Leaky" cell membranes due to suboptimal culture conditions or handling. Keep cell densities low ($<1.5 \times 10^6$ cells/ml) and feed cells with fresh medium. Avoid large temperature fluctuations in culture medium or wash buffers. Avoid vigorous pipetting when resuspending cell pellets and keep centrifugation force $\leq 250 \times g$.
Low % cytotoxicity observed with cell-mediated cytotoxicity assay	Percent cytotoxicity was too low for convenient quantitation. To increase % cytotoxicity, increase the incubation time with the cytotoxic cells (Section 5.B) from 4 hours to 6–8 hours. Do not incubate overnight because cell proliferation may lead to inaccurate results.
Absorbance values above linear range of plate reader	Bubbles were present in wells of plate. Gently break bubbles with a syringe needle, and repeat absorbance readings.
	Too much LDH activity. Repeat assay and shorten LDH reaction time (Section 5.B, Step 8) to 15–20 minutes.
Low overall absorbance values	Plate reader was set at incorrect wavelength. Set plate reader to 490nm or 492nm, and repeat absorbance readings.
	Substrate was degraded by light. Check that substrate preparation and LDH reaction (Section 5.B, Steps 7 and 8) are performed while protected from light.
Low absorbance for Target Cell Maximum Release	Target cell number was not optimized. Optimize the number of target cells added to the assay (Section 5.A).

9. References

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10. Appendix

10.A. Composition of Buffers and Solutions

PBS + 1% BSA

0.2g/L	KCl
8.0g/L	NaCl
0.2g/L	KH_2PO_4
1.15g/L	Na_2HPO_4
1% (w/v)	bovine serum albumin

Dissolve in deionized water, and filter-sterilize before use.

Lysis Solution (10X)

9% (v/v)	Triton® X-100

Stop Solution

1M	acetic acid

10.B. Related Products

Viability Assays

Product	Size	Cat.#
RealTime-Glo™ MT Cell Viability Assay	100 reactions	G9711
CellTiter-Glo® 2.0 Assay	10ml	G9241
CellTiter-Glo® Luminescent Cell Viability Assay	10ml	G7570
CellTiter-Glo® 3D Cell Viability Assay	10ml	G9681
CellTiter-Fluor™ Cell Viability Assay	10ml	G6080
CellTiter-Blue® Cell Viability Assay	20ml	G8080
CellTiter 96® AQ_{ueous} One Solution Cell Proliferation Assay	1,000 assays	G3580
CellTiter 96® AQ_{ueous} MTS Reagent Powder	250mg	G1112
CellTiter 96® Non-Radioactive Cell Proliferation Assay	1,000 assays	G4000

Available in additional sizes.

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10.B.Related Products (continued)

Cytotoxicity Assays

Product	Size	Cat.#
CellTox™ Green Cytotoxicity Assay	10ml	G8741
CytoTox-Glo™ Cytotoxicity Assay	10ml	G9290
CytoTox-Fluor™ Cytotoxicity Assay	10ml	G9260
CytoTox-ONE™ Homogeneous Membrane Integrity Assay	200–800 assays	G7890

Available in additional sizes.

Apoptosis Assays

Product	Size	Cat.#
Caspase-Glo® 3/7 Assay*	10ml	G8091
Caspase-Glo® 8 Assay*	10ml	G8201
Caspase-Glo® 9 Assay*	10ml	G8211
Apo-ONE® Homogeneous Caspase-3/7 Assay	10ml	G7790

*Available in additional sizes.

Multiplexed Viability and Cytotoxicity Assays

Product	Size	Cat.#
MultiTox-Glo Multiplex Cytotoxicity Assay	10ml	G9270
MultiTox-Fluor Multiplex Cytotoxicity Assay	10ml	G9200

Available in additional sizes.

Mechanism-Based Viability and Cytotoxicity Assays

Product	Size	Cat.#
ApoTox-Glo™ Triplex Assay	10ml	G6320
ApoLive-Glo™ Multiplex Assay	10ml	G6410
Mitochondrial ToxGlo™ Assay	10ml	G8000

Available in additional sizes.

Metabolism Assays

Product	Size	Cat.#
NAD(P)H-Glo™ Detection System	10ml	G9061
NAD/NADH-Glo™ Assay	10ml	G9071
NADP/NADPH-Glo™ Assay	10ml	G9081

Other sizes are available.

Mitochondrial Toxicity Assay

Product	Size	Cat.#
Mitochondrial ToxGlo™ Assay	10ml	G8000
	100ml	G8001

Oxidative Stress Assays

Product	Size	Cat.#
ROS-Glo™ H_2O_2 Assay	10ml	G8820
GSH-Glo™ Glutathione Assay	10ml	V6911
GSH/GSSG-Glo™ Assay	10ml	V6611

Other sizes are available.

Cytochrome P450 Cell-Based Assays

Product	Size	Cat.#
P450-Glo™ CYP1A2 Induction/Inhibition Assay	10ml	V8421
P450-Glo™ CYP3A4 Assay with Luciferin-IPA	10ml	V9001
P450-Glo™ CYP2C9 Assay	10ml	V8791

Other sizes are available.

Detection Instrumentation

Product	Size	Cat.#
GloMax® Discover System	1 each	GM3000
GloMax® Explorer System	1 each	GM3500

11. Summary of Changes

The following change was made to the 7/16 revision of this document:

1. The name of the Vehicle-Only Cells Control was clarified in the Maximum LDH Release Control listed in Section 4.A.