Technical Bulletin

CytoTox 96® Non-Radioactive Cytotoxicity Assay

INSTRUCTIONS FOR USE OF PRODUCT G1780.

www.promega.com

PRINTED IN USA.
Revised 12/12

Part# TB163

CytoTox 96® Non-Radioactive Cytotoxicity Assay

All technical literature is available on the Internet at www.promega.com/protocols Please visit the web site to verify that you are using the most current version of this Technical Bulletin. Please contact Promega Technical Services if you have questions on use of this system. E-mail: techserv@promega.com

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. Released LDH in culture supernatants is measured with a 30-minute coupled enzymatic assay, which results in the conversion of a tetrazolium salt (INT) into a red formazan product. The amount

1. Description (continued)

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 determining LDH using tetrazolium salts in conjunction with diaphorase or alternate electron acceptors have been used for several 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 as follows:

$$\text{NAD}^+ + \text{lactate} \xrightarrow{\textbf{LDH}} \text{pyruvate} + \text{NADH}$$

$$\text{NADH} + \text{INT} \xrightarrow{\textbf{Diaphorase}} \text{NAD+} + \text{formazan (red)}$$

Applications of the CytoTox 96® Assay

- cell-mediated cytotoxicity (4)
- cytotoxicity mediated by chemicals or other agents (5–8)
- total cell number (9)

Advantages of the CytoTox 96® Assay

- eliminates labeling of cells before experiment
- eliminates paperwork and safety issues of radioactivity
- allows use of standard plate reader
- can reveal early, low-level cytotoxicity

Selected Citations Using the CytoTox 96® Assay

- Spagnou, S., Miller, A.D. and Keller, M. (2004) Lipidic carriers of siRNA: Differences in the formulation, cellular uptake, and delivery with plasmid DNA. *Biochemistry* **43**, 13348–56.

  The CytoTox 96® Non-Radioactive Cytotoxicity Assay was used to determine the cytotoxic effect of lipophilic transfection reagents commonly used for siRNA transfection. Data are presented as the percent cell death observed in HeLa and IGROV-1 cells at 24 hours post-transfection.

- Hernández, J.M. *et al.* (2003) Novel kidney cancer immunotherapy based on the granulocyte-macrophage colony-stimulating factor and carbonic anhydrase IX fusion gene. *Clin. Cancer Res.* **9**, 1906–16.

  The CytoTox 96® Non-Radioactive Cytotoxicity Assay was used to determine specific cytotoxicity of human dendritic cells transduced with adenoviruses encoding a fusion protein of granulocyte-macrophage colony stimulating factor and carbonic anhydrase IX.

For additional peer-reviewed articles that cite use of the CytoTox 96® Assay, visit:
www.promega.com/citations/

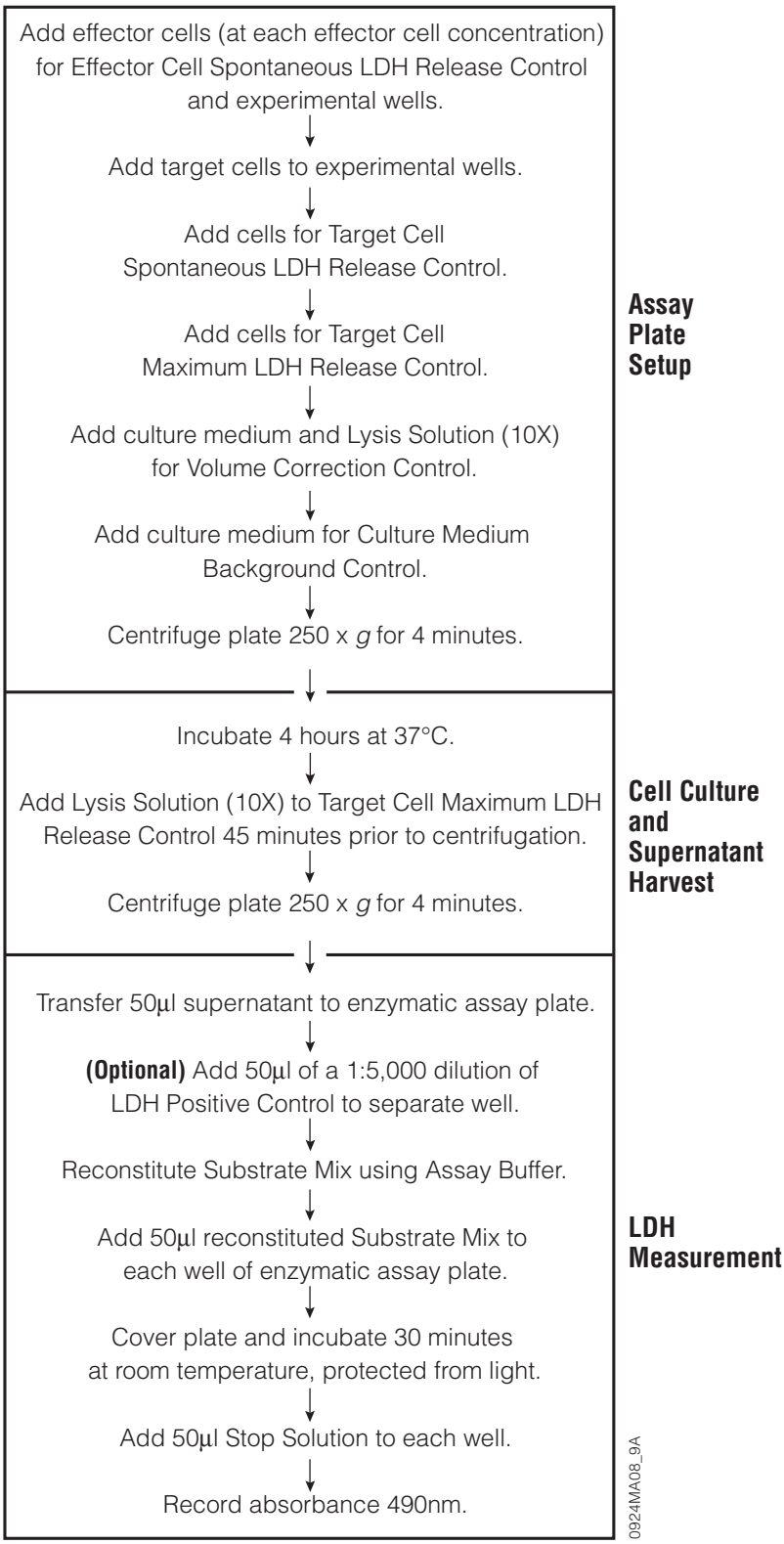

Figure 1. CytoTox 96® Non-Radioactive Cytotoxicity Assay procedure for cell-mediated cytotoxicity using effector/target cells.

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 the cell-mediated cytotoxicity assay protocol for 1,000 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. Reconstituted Substrate Mix may be stored for 6–8 weeks at –20°C 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. Twelve milliliters of the supernatant can then be used to reconstitute the substrate mix.

3. General Considerations

3.A. Background Absorbance Corrections

Two factors in tissue culture medium can contribute to background absorbance using the CytoTox 96® Assay: phenol red from medium and LDH from animal sera. Background absorbance from both 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.D). 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.

3.B. CytoTox 96® Assay Controls

The five controls listed below must be performed with the CytoTox 96® Assay. 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.

4. 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 using your target cell population(s) to determine the optimum number of target cells to use with the CytoTox 96® Assay and to ensure an adequate signal-to-noise ratio (Figure 2). The LDH Positive Control supplied may be used to verify that the LDH assay is functioning properly.

Materials to Be Supplied by the User
(Solution composition is provided in Section 9.A.)

- round- or V-bottom 96-well tissue culture plates
- multichannel pipettor
- **Optional:** PBS + 1% BSA (bovine serum albumin)

4.A. Assay Plate Setup

1. Prepare serial dilutions of each target 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.

4.A. Assay Plate Setup (continued)

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. Triplicate or quadruplicate wells are recommended.

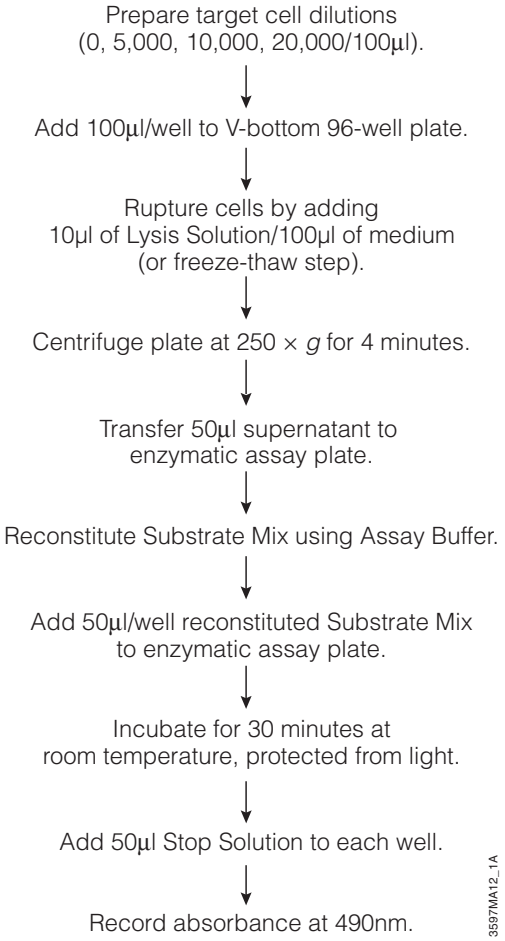

Figure 2. Optimization of target cell number.

4.B. Cell Lysis and Supernatant Harvest

1. Add 10µl of Lysis Solution (10X) per 100µl of medium to all wells to lyse cells.
2. Incubate for 45 minutes in a humidified chamber at 37°C, 5% CO_2.

   Note: As an alternative to Steps 1 and 2, lyse the cells by incubating the plate at –70°C for approximately 30 minutes followed by thawing at 37°C for 15 minutes. Proceed to Step 3.
3. Centrifuge the plate at 250 × *g* for 4 minutes.

4.C. LDH Measurement

1. Transfer 50µl aliquots from all wells to a fresh 96-well flat-bottom (enzymatic assay) plate.
2. Thaw the Assay Buffer, remove 12ml and promptly store the unused portion at –20°C. A 37°C water bath may be used to thaw the Assay Buffer, but it should not be left at 37°C longer than necessary.

   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. Twelve milliliters of the supernatant can then be used to reconstitute the substrate mix.

   Warm the 12ml of Assay Buffer to room temperature (keep protected from light). Add the 12ml of room temperature Assay Buffer to a bottle of Substrate Mix. Invert and shake gently to dissolve the substrate. One bottle will supply enough substrate for two 96-well plates. Once resuspended, protect the substrate from strong direct light and use immediately.
3. Add 50µl of the reconstituted Substrate Mix to each well of the plate. Cover the plate with foil or a small opaque box to protect it from light. Incubate at room temperature for 30 minutes.

   Note: Store unused portions of the reconstituted Substrate Mix tightly capped at –20°C for ≤6–8 weeks.
4. Add 50µl of Stop Solution to each well.
5. Pop any large bubbles using a syringe needle, and record the absorbance at 490 or 492nm within one hour after the addition of Stop Solution.
6. Determine the concentration of target cells yielding absorbance values at least two times the background absorbance of the medium control.

   Note: If you normally co-culture 100µl/well of target cells with 100µl/well of 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 LDH is increased.

5. Cell-Mediated Cytotoxicity Assay

5.A. Assay Plate Setup

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.

1. **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 in the experimental wells (use medium alone with no cells to bring up the volume).
2. **Experimental Wells:** Add a constant number of target cells (determined in Section 4) 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 should be a minimum of 100µl/well.
3. **Target Cell Spontaneous LDH Release:** Add target cells (concentration determined in Section 4) to a triplicate or quadruplicate set of wells containing culture medium. The final volume must be the same as the experimental wells containing both target and effector cells (use culture medium to adjust volume).
4. **Target Cell Maximum LDH Release:** Add target cells (concentration determined in Section 4) to a triplicate or quadruplicate set of wells containing culture medium. The final volume must be the same as 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 presence of Lysis Solution for 45 minutes prior to harvesting the supernatants.
5. **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.
6. **Culture Medium Background:** 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.
7. **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 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. Triplicate or quadruplicate wells are recommended.

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

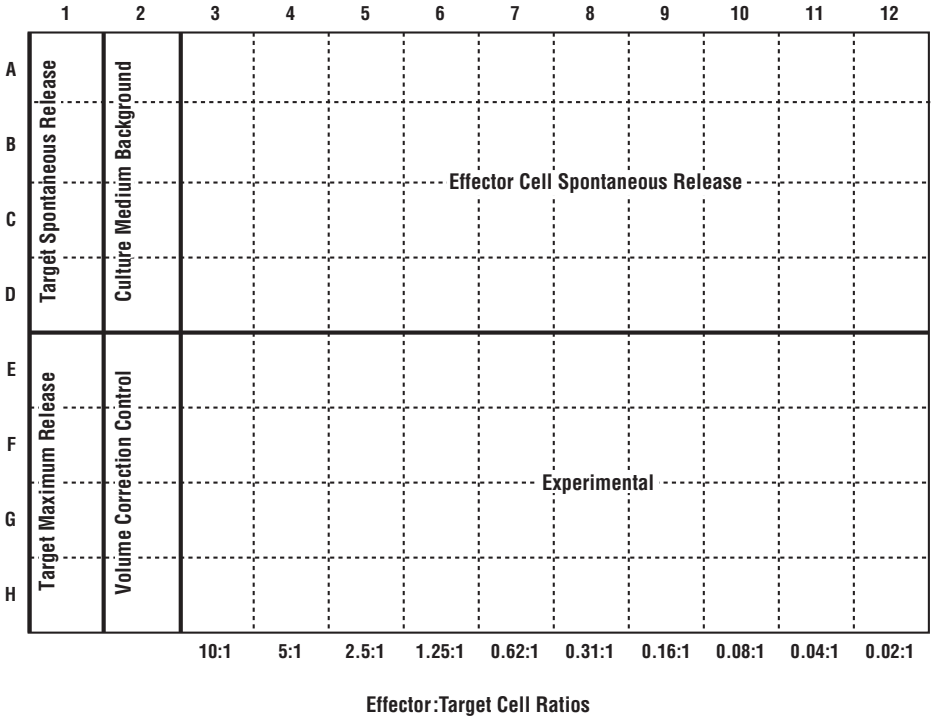

Figure 3. Representative CytoTox 96® Non-Radioactive Cytotoxicity Assay plate setup. The drawing is a representation of a 96-well plate containing the experimental and control wells necessary for the CytoTox 96® Assay. Perform each experimental and control reaction in triplicate or quadruplicate.

5.B. Cell Culture and Supernatant Harvest

1. 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.

2. 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).

3. After the 4-hour incubation, centrifuge the plate at 250 × *g* for 4 minutes.

5.C. LDH Measurement

1. Transfer 50µl aliquots from all wells using a multichannel pipettor to a fresh 96-well flat-bottom (enzymatic assay) plate.
2. Thaw the Assay Buffer, remove 12ml and promptly store the unused portion at –20°C. A 37°C water bath may be used to thaw the Assay Buffer, but it should not be left at 37°C longer than necessary.

   Warm the 12ml of Assay Buffer to room temperature (keep protected from light). Add the 12ml of room temperature Assay Buffer to one bottle of Substrate Mix. Invert and shake gently to dissolve the Substrate Mix. One bottle will supply enough substrate for two 96-well plates. Once resuspended, protect the Substrate from strong direct light and use immediately.
3. Add 50µl of reconstituted Substrate Mix to each well of the enzymatic assay plate containing samples transferred from the cytotoxicity assay plate. 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 reconstituted Substrate Mix tightly capped at –20°C for ≤6–8 weeks.
4. Add 50µl of Stop Solution to each well.
5. Pop any large bubbles using a syringe needle, and record the absorbance at 490 or 492nm within 1 hour after the addition of Stop Solution.

5.D. Calculation of Results

1. Subtract the average of absorbance values of the Culture Medium Background from all absorbance values of Experimental, Target Cell Spontaneous LDH Release and Effector Cell Spontaneous LDH Release.
2. Subtract the average of the absorbance values of 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 compute percent cytotoxicity for each effector:target cell ratio.

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

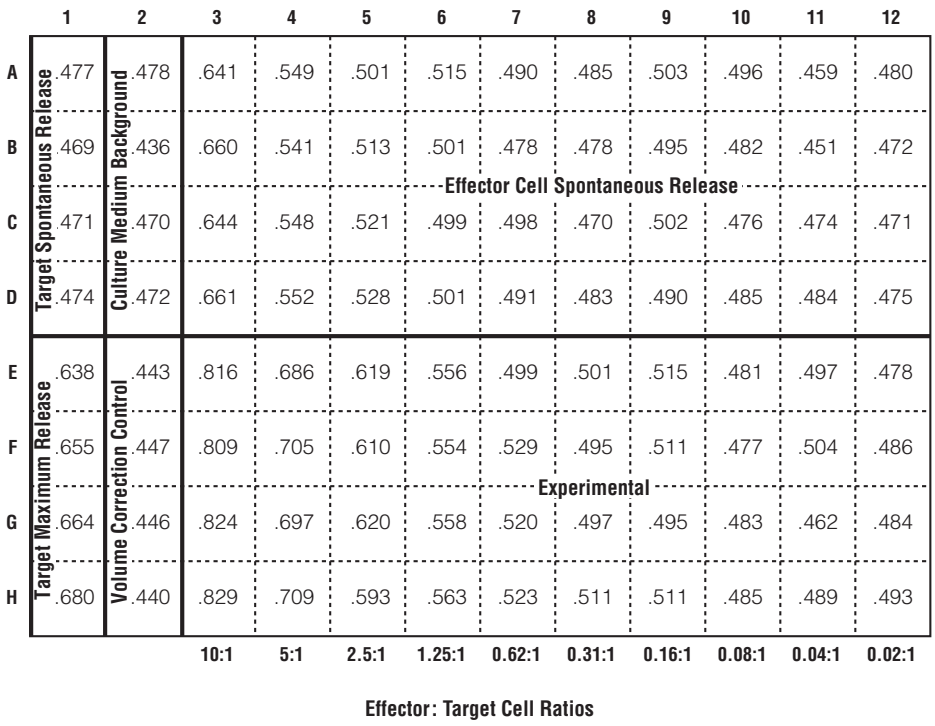

Figure 4. Representative data from a CytoTox 96® Non-Radioactive Cytotoxicity Assay performed at Promega. The assay conditions used to generate these data are described previously in this section.

Sample Calculation

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.

Sample Calculation (continued)

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. Assays Using A Single Population of Cells

6.A. Total Cell Number Assay

The CytoTox 96® Assay indirectly measures the 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. Certain detergents (SDS and cetrimide) have been shown to 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 9.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. Reconstituted Substrate Mix (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 the Stop Solution. The plate can be read at 490nm using an ELISA 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." Figure 5 summarizes these steps.

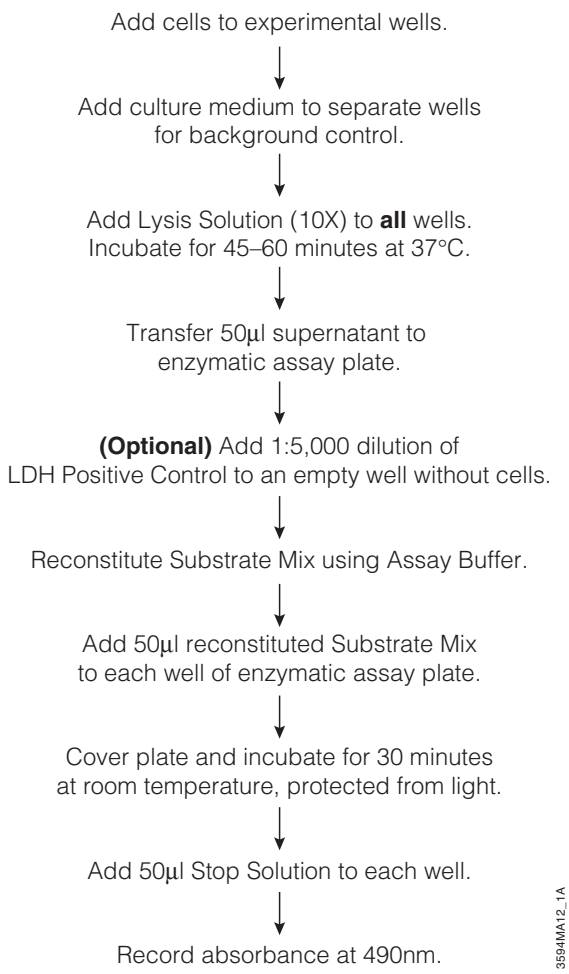

Figure 5. Modified CytoTox 96® Non-Radioactive Cytotoxicity Assay procedure for detecting total cell number.

6.B. Cytotoxicity Assay

The CytoTox 96® Assay can also be used to measure death of a single cell type (i.e., without an effector cell) in culture, such as after treatment with a cytotoxic drug (10). An example protocol, where the CytoTox 96® Assay was used to measure cell death initiated by transfected N-methyl-D-aspartate (NMDA) receptors (11), is given in Figure 6.

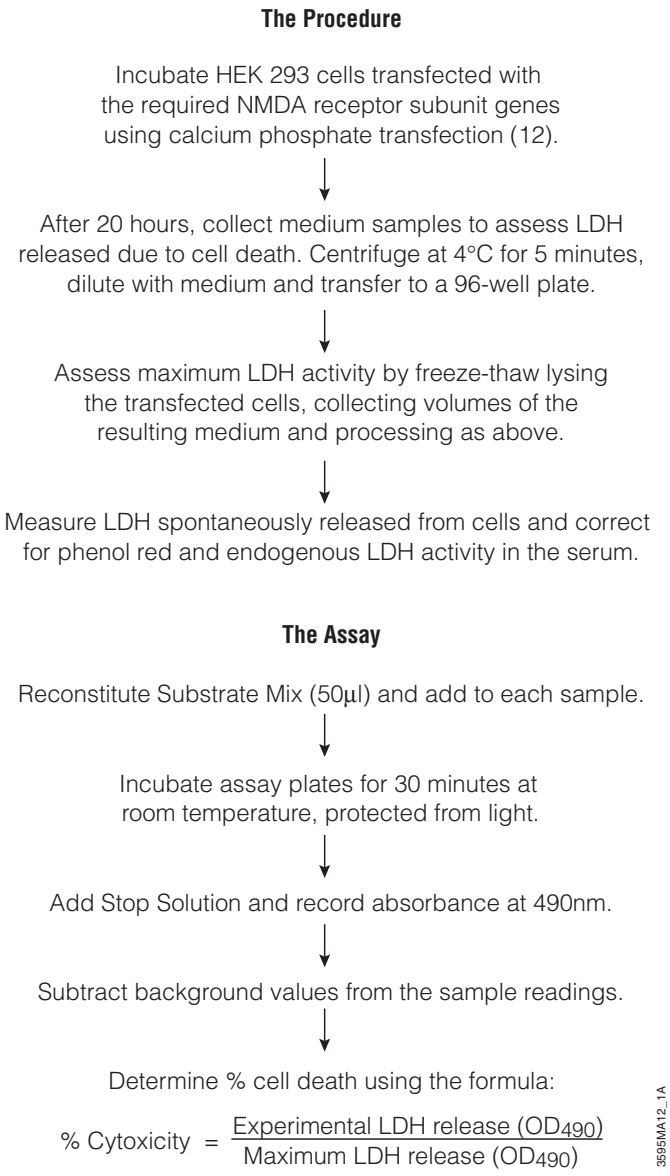

Figure 6. Modified CytoTox 96® Non-Radioactive Cytotoxicity Assay to measure cell death initiated by transfected NMDA receptors (11) (cytotoxicity using one cell type).

7. 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. The use of 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 values 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 fed 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	Percent cytotoxicity too low for convenient quantitation. In cases where it is desirable 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 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.C, Step 3) to 15–20 minutes.

7. Troubleshooting (continued)

Symptoms	Causes and Comments
Low overall absorbance values	Plate reader set at incorrect absorbance. Set plate reader to 490 or 492nm and repeat absorbance readings.
	Substrate degraded by light. Check that the substrate preparation and LDH reaction (Section 5.C, Steps 2 and 3) are performed while protected from light.
Low value for Target Cell Maximum Release	Target cell number not optimized. Optimize the number of target cells added to the assay (Section 4).

8. References

1. Nachlas, M.M. *et al.* (1960) The determination of lactic dehydrogenase with a tetrazolium salt. *Anal. Biochem.* **1**, 317–26.
2. Korzeniewski, C. and Callewaert, D.M. (1983) An enzyme-release assay for natural cytotoxicity. *J. Immunol. Meth.* **64**, 313–20.
3. Decker, T. and Lohmann-Matthes, M.L. (1988) A quick and simple method for the quantitation of lactate dehydrogenase release in measurements of cellular cytotoxicity and tumor necrosis factor (TNF) activity. *J. Immunol. Meth.* **115**, 61–9.
4. Brander, C. *et al.* (1993) Carrier-mediated uptake and presentation of a major histocompatibility complex class I-restricted peptide. *Eur. J. Immunol.* **23**, 3217–23.
5. Behl, C. *et al.* (1994) Hydrogen peroxide mediates amyloid beta protein toxicity. *Cell* **77**, 817–27.
6. Lappalainen, K. *et al.* (1994) Comparison of cell proliferation and toxicity assays using two cationic liposomes. *Pharm. Res.* **11**, 1127–31.
7. Allen, M.J. and Rushton, N. (1994) Use of the CytoTox 96™ Assay in routine biocompatibility testing in vitro. *Promega Notes* **45**, 7–10.
8. Sinensky, M.C., Leiser, A.L. and Babich, H. (1995) Oxidative stress aspects of the cytotoxicity of carbamide peroxide: in vitro studies. *Toxicol. Lett.* **75**, 101–9.
9. Moravec, R. (1994) Total cell quantitation using the CytoTox 96™ Non-Radioactive Cytotoxicity Assay. *Promega Notes* **45**, 11–12.
10. Singer, C.A. *et al.* (1999) The mitogen-activated protein kinase pathway mediates estrogen neuroprotection after glutamate toxicity in primary cortical neurons. *J. Neurosci.* **19**, 2455–63.
11. Miroslav, C. *et al.* (1995) Using Promega's CytoTox 96® Non-Radioactive Cytotoxicity Assay to measure cell death mediated by NMDA receptor subunits. *Promega Notes* **51**, 21–22.
12. Gorman, C.M., Gies, D.R. and McCray, G. (1990) Transient production of proteins using an adenovirus transformed cell line. *DNA Prot. Eng. Technol.* **2**, 3.

9. Appendix

9.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

9.B. Related Products

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.

Viability Assays

Product	Size	Cat.#
CellTiter-Glo® Luminescent Cell Viability Assay*	10 × 100ml	G7571
CellTiter-Fluor™ Cell Viability Assay*	10ml	G6080
CellTiter-Blue® Cell Viability Assay*	100ml	G8081
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.

9.B. Related Products (continued)

Cytotoxicity Assays

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

*Available in additional sizes.

Apoptosis Assays

Product	Size	Cat.#
Caspase-Glo® 2 Assay*	10ml	G0940
Caspase-Glo® 3/7 Assay*	10ml	G8091
Caspase-Glo® 6 Assay*	10ml	G0970
Caspase-Glo® 8 Assay*	10ml	G8201
Caspase-Glo® 9 Assay*	10ml	G8211
Apo-ONE® Homogeneous Caspase-3/7 Assay	100ml	G7791

*Available in additional sizes.