FREQUENTLY ASKED QUESTIONS
CYTOTOX-ONE™ HOMOGENEOUS MEMBRANE INTEGRITY ASSAY

BY ABIGAIL FARFAN, M.A., AND TRISTA SCHAGAT, PH.D., PROMEGA CORPORATION

Promega Corporation has released the CytoTox-ONE™ Homogeneous Membrane Integrity Assay in order to assist researchers interested in measuring cytotoxicity in vitro in a homogeneous format. This article addresses questions about the general assay protocol, assay characteristics, effects of test compounds on assay results, and the ability to multiplex this assay with other cell-based assays

What is the CytoTox-ONE™ Homogeneous Membrane Integrity Assay, and how does it differ from CytoTox 96® Non-Radioactive Cytotoxicity Assay?

The CytoTox-ONE™ Homogeneous Membrane Integrity Assay[a] is a homogeneous, fluorometric method for estimating the number of nonviable cells present in multiwell plates. The CytoTox-ONE™ Reagent is prepared from the supplied Substrate Mix and Assay Buffer, equilibrated to room temperature and added to cells. Results can be read after mixing and a 10-minute incubation. Like the CytoTox 96® Assay, the CytoTox-ONE™ Assay measures the release of lactate dehydrogenase (LDH) from cells that have a damaged plasma membrane. Because the CytoTox-ONE™ Reagent does not damage healthy cells, you can add it directly in assay wells containing a mixed population of viable and damaged cells. LDH released into the culture medium is measured with a 10-minute coupled enzymatic assay that results in the conversion of resazurin to a fluorescent resorufin product (1). This is in contrast to the CytoTox 96® Assay, which is a 30-minute colorimetric assay that requires the transfer of medium from the cell culture assay plate to a second plate.

What media are compatible with the CytoTox-ONE™ Assay?

Because the CytoTox-ONE™ Assay measures LDH released by dead cells into medium, and the medium contains varying amounts of LDH, you need to perform a "no-cell" control in which the CytoTox-ONE™ Reagent is added to medium alone. This control will determine the background fluorescence that the medium contributes to the assay. This background value can be subtracted from test sample values. The CytoTox-ONE™ Assay is compatible with standard cell culture media containing phenol red and amino acid supplements.

Serum and other medium supplements can also affect background or results. See the *CytoTox-ONE™ Assay Technical Bulletin* #TB306 (1) for more details.

I do not have a filter set of 560nm and 590nm for my fluorometer. What can I do?

Although the optimal excitation and emission wavelengths for the fluorescent resorufin product are 560nm and 590nm, respectively, you may use excitation filters in the 530–570nm range and emission filters in the 580–620nm range (2). Using suboptimal excitation and emission wavelengths may result in reduced sensitivity.

Is it possible to perform other assays in combination with the CytoTox-ONE™ Assay (multiplex assays)?

A portion of the medium from each well may be transferred to a separate multiwell plate to measure LDH release using the CytoTox-ONE™ Assay. A separate assay can be performed on the sample remaining in the original well. Some examples of this application include estimating the number of viable cells by measuring the ATP content using the CellTiter-Glo® Assay[a] or by measuring tetrazolium reduction using the CellTiter 96® AQ_{ueous} One Solution Assay[b]. Alternatively, the level of apoptosis could be determined by measuring the caspase activity using the Apo-ONE® Homogeneous Caspase-3/7 Assay[c] (1).

How is resazurin reduced in a cytotoxicity assay versus a cell viability assay?

Resazurin is a component of both the CytoTox-ONE™ Homogeneous Membrane Integrity Assay and the CellTiter-Blue™ Cell Viability Assay, but in different formulations.

The CytoTox-ONE™ Homogeneous Membrane Integrity Assay measures the release of lactate dehydrogenase (LDH) into the culture medium from cells with damaged membranes. LDH release results in the conversion of resazurin into resorufin using an in vitro coupled enzymatic assay (1).

Using the reaction conditions recommended in the CytoTox-ONE™ Homogeneous Membrane Integrity Assay protocol, a 10-minute incubation at ambient temperature results in a negligible amount of resazurin reduction by the viable cell population and only a very slight increase in fluorescence in the control viable cells in fresh serum-free medium (3). The rate of the CytoTox-ONE™ reaction is

increased by providing excess substrates, pyruvate, NAD+ and diaphorase.

The CellTiter-Blue™ Cell Viability Assay is not an in vitro coupled enzymatic reaction. Instead, conversion of resazurin to resorufin takes place by metabolically active cells, resulting in the generation of a fluorescent product that is proportional to the cell number. Nonviable cells rapidly lose metabolic capacity, thus do not reduce the indicator dye, and do not generate a fluorescent signal. The reaction occurs at 37°C for a period of incubation of 1 to 4 hours. ■

References

1. *CytoTox-ONE™ Homogeneous Membrane Integrity Assay Technical Bulletin* #TB306, Promega Corporation.
2. Hoffman, R. (2002) *Cell Notes* **4**, 10–11.
3. *CellTiter-Blue™ Cell Viability Assay Technical Bulletin*, #TB317, Promega Corporation.

Protocol

CytoTox-ONE™ Homogeneous Membrane Integrity Assay Technical Bulletin #TB306
(www.promega.com/tbs/tb306/tb306.html)

Ordering Information

Product	Size	Cat.#
CytoTox-ONE™ Homogeneous Membrane Integrity Assay[a]	200–800 assays	G7890
	1,000–4,000 assays	G7891
CytoTox-ONE™ Homogeneous Membrane Integrity Assay, HTP[a]	1,000–4,000 assays	G7892

[a]Patent Pending.

[b]The MTS tetrazolium compound is the subject of U.S. Pat. No. 5,185,450 assigned to the University of South Florida and is licensed exclusively to Promega Corporation.

[c]This product is covered by U.S. Pat. Nos. 4,557,862 and 4,640,893 and is sold for research use only. All other uses, including but not limited to use as a clinical diagnostic or therapeutic, require a separate license. Please contact Promega Corporation for details relating to obtaining a license for such other use.

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Promega Corporation
2800 Woods Hollow Road
Madison, WI 53711-5399 USA
Tel: 608-274-4330
Fax: 608-277-2516
Toll-Free: 800-356-9526
Toll-Free Fax: 800-356-1970
Internet: www.promega.com

Promega Biosciences, Inc.
A Division of Promega Corporation
San Luis Obispo, California.

***Australia,* Sydney**
Tel: 02 9565 1100
Fax: 02 9550 4454
Free Call: 1 800 225 123
Free Fax: 1 800 626 017
E-mail: aus_custserv@au.promega.com

***China,* Beijing**
Tel: 10 6849 8287
Fax: 10 6849 8390
E-mail: promega@promega.com.cn

***France,* Lyon**
Tél: 04 37 22 50 00
Fax: 04 37 22 50 10
Numéro Vert: 0 800 48 79 99
E-mail: fr_custserv@fr.promega.com

***Germany/Austria,* Mannheim**
Tel: (+49) (0) 621 8501 0
Fax: (+49) (0) 621 8501 222
Free Tel: 00800 77663422
Free Fax: 00800 77663423
E-mail: de_custserv@de.promega.com

***Italy,* Milan**
Tel: 02 5405 0194
Fax: 02 5518 5664
Numero Verde: 800 69 1818
E-mail: it_custserv@it.promega.com

***Japan,* Tokyo**
Tel: 03 3669 7981
Fax: 03 3669 7982
E-mail: prometec@jp.promega.com

***Belgium/ Luxembourg/ The Netherlands,* Leiden**
Tel: (+31) (0) 71 5324244
Fax: (+31) (0) 71 5324907
Tel BE: 0800 18098
Fax BE: 0800 16971
Free Tel NL: 0800 0221910
Free Fax NL: 0800 0226545
E-mail: bnl_custserv@nl.promega.com

***Pacific Asia Region,* Singapore**
Tel: 65 6254 5265
Fax: 65 6254 8645
E-mail: nng@promega.com

***Switzerland,* Wallisellen**
Tel: 01 878 90 00
Fax: 01 878 90 10
Technical Service: 01 878 90 20
E-mail: catalys_custserv@catalys.promega.com

***United Kingdom,* Southampton**
Tel: 023 8076 0225
Fax: 023 8076 7014
Free Phone: 0800 378994
Free Fax: 0800 181037
E-mail: ukcustserve@uk.promega.com