CHOOSING THE RIGHT CELL-BASED ASSAY FOR YOUR RESEARCH

BY TERRY RISS, PH.D., MARTHA O'BRIEN, PH.D., AND RICHARD MORAVEC, B.S., PROMEGA CORPORATION

A variety of methods for measuring in vitro toxicity and apoptosis have been adapted for use as automated high-throughput screeening assays. Choosing the right assay tool and the appropriate endpoint to measure are essential to generate meaningful data. We discuss examples of homogeneous in vitro cytotoxicity and apoptosis assays that measure different endpoints and describe the considerations for using each assay.

Introduction

Choosing a cell viability or cytotoxicity assay from among the many different options available can be a challenging task. Picking the best assay format to suit particular needs requires understanding what each assay is measuring as an endpoint, how the measurement correlates with cell viability, and what the limitations of the assay chemistries are.

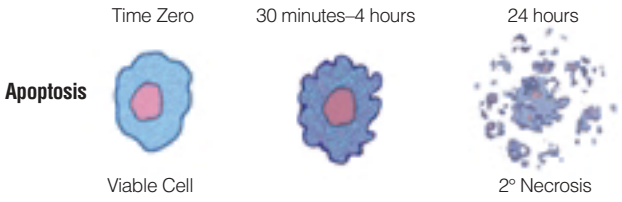
Here we provide recommendations for characterizing a model assay system and some of the factors to consider when choosing cell-based assays for automated systems. We also describe the broad portfolio of homogeneous, cell-based assays for viability, cytotoxicity, and apoptosis available from Promega, highlighting the considerations for using each assay.

Establishing an In Vitro Model System

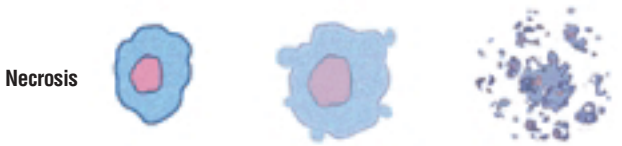
The species of origin and cell types used in cytotoxicity studies are often dictated by specific project goals or the drug target that is being investigated. Regardless of the model system chosen, establishing a consistent and reproducible procedure for setting up assay plates is important. The number of cells per well and the equilibration period prior to the assay may affect cellular physiology. Maintenance and handling of stock cultures at each step of the manufacturing process should be standardized and validated for consistency. Assay responsiveness to test compounds can be influenced by many subtle factors including culture medium surface-to-volume ratio, gas exchange, evaporation of liquids, and edge effects. These factors are especially important considerations when attempting to scale-up assay throughput by changing from 96- to 384- or 1536-well formats.

Choosing an Endpoint to Measure

One of the first things to decide before choosing an assay format is exactly what information you want to measure at the end of a treatment period. Assays are available to measure a variety of different markers that indicate the number of dead cells (cytotoxicity assay), the number of live cells (viability assay), the total number of cells, or the mechanism of cell death (e.g., apoptosis). Table 1 shows a comparison of Promega's homogeneous cell-based assays and lists the measured parameters, sensitivity of detection, incubation time, and detection method.



LDH Release	0	+	+++
Caspase	0	+++	++
ATP	+++	++	0
MTS	+++	++	0
Resazurin	+++	++	0



LDH Release	0	+++	++
Caspase	0	0	0
ATP	+++	0	0
MTS	+++	0	0
Resazurin	+++	0	0

3863MA10_2B

Figure 1. Mechanisms of cell death can be determined by measuring different markers of cell viability and apoptosis in vitro.

A basic understanding of the changes that occur during different mechanisms of cell death will help in deciding which endpoint to choose for a cytotoxicity assay. Figure 1 shows a simplified example illustrating chronological changes occurring during apoptosis and necrosis and the results that would be expected from using the assays listed in Table 1 to measure different markers.

Cultured cells undergoing apoptosis in vitro eventually undergo secondary necrosis. After extended incubation, apoptotic cells ultimately shut down metabolism, lose membrane integrity and release their cytoplasmic contents into the culture medium. Markers of apoptosis such as

Table 1. Comparison of Promega Cell Viability Assays.

	CellTiter-Glo® Luminescent Cell Viability Assay (ATP)	CellTiter-Blue™ Cell Viability Assay	CellTiter 96® AQ_{ueous} One Solution Assay	CytoTox-ONE™ Membrane Integrity Assay	Apo-ONE® Homogeneous Caspase-3/7 Assay	Caspase-Glo™ 3/7 Assay
Incubation Time	10 minutes	1–4 hours	1–4 hours	10 minutes	1–18 hours	30 minutes–2 hours
Homogeneous Protocol	Yes	Yes	Yes	Yes	Yes	Yes
Parameter Measured	ATP	Resazurin (Reducing Potential)	MTS (Reducing Potential)	LDH Release	Caspase Activity	Caspase Activity
Detection Method	Bioluminescent	Fluorometric/Colorimetric	Colorimetric	Fluorometric	Fluorometric	Bioluminescent
Sensitivity*						
96-well format	50 cells	390 cells**	800 cells	800 cells	Several hundred cells in a population	20 cells
384-well format	15 cells	50 cells**	200 cells	200 cells	Several hundred cells in a population	20 cells

*Sensitivity is dependent on cell type and other experimental conditions. **Measured by fluorescent readout.

caspase activity may be expressed only transiently. Therefore, to determine if apoptosis is the primary mechanism of cell death, understanding the kinetics of the cell death process in your model system is critical.

Cells undergoing necrosis typically show rapid swelling, lose membrane integrity, shut down metabolism and release their cytoplasmic contents into the surrounding culture medium. Cells undergoing rapid necrosis in vitro do not have sufficient time or energy to activate apoptotic machinery and will not express apoptotic markers.

If the information sought from the assay is simply whether there is a difference between "no treatment" negative controls and "toxin-treatment" of experimental wells, the choice between measuring the number of viable cells or the number of dead cells may be irrelevant. However, if more detailed information on the mechanism of cell death is desired, the duration of exposure to toxin, the concentration of the test compound, and the choice of the assay endpoint become critical.

Characterizing Assay Responsiveness

Protocols used to measure cytotoxicity in vitro differ widely (Figure 2). Often assay plates are set up containing cells and allowed to equilibrate for a predetermined period before adding test compounds. Alternatively, cells may be added directly to plates that already contain library compounds. The duration of exposure to the toxin may vary from less than an hour to several days, depending on specific project goals.

Figure 2. Generalized scheme representing an in vitro cytotoxicity assay protocol.

Brief periods of exposure may be used to determine if test compounds cause an immediate necrotic insult to cells, whereas exposure for several days is commonly used to determine if test compounds cause an inhibition of cell proliferation.

Cell viability or cytotoxicity measurements usually are determined at the end of the exposure period. Assays that require only a few minutes to generate a measurable signal (e.g., ATP quantitation or LDH-release assays) provide information representing a snapshot in time and have an advantage over assays that may require several hours of incubation to develop a signal (e.g., MTS or resazurin). In addition to being more convenient, rapid assays reduce the chance of artifacts caused by interaction of test compounds with assay chemistry.

In vitro cultured cells exist as a heterogeneous population. When populations of cells are exposed to test compounds they do not all respond simultaneously. Cells exposed to toxin may respond over the course of several hours or days, depending on many factors including the mechanism of cell death, the concentration of the toxin, and the duration of exposure. As a result of culture heterogeneity, the data from most plate-based assay formats represent an average of the signal from the population of cells.

Determining Dose and Duration of Exposure

Characterizing assay responsiveness for each in vitro model system is important, especially when trying to distinguish between different mechanisms of cell death. Initial characterization experiments should include a determination of the appropriate assay window using an established positive control.

Figures 3 and 4 show the results of two characterization experiments to determine the kinetics of cell death caused by different concentrations of tamoxifen treatment of HepG2 cells. The two experiments measured different endpoints: ATP as an indicator of viable cells and caspase activity as a marker for apoptotic cells.

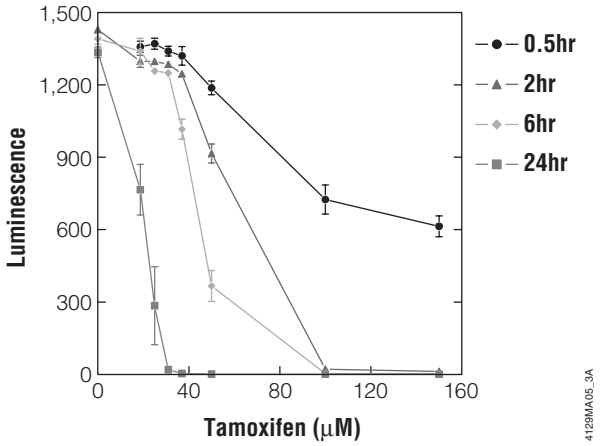


Figure 3. Characterization of the toxic effects of tamoxifen on HepG2 cells using the CellTiter-Glo® Luminescent Cell Viability Assay to measure ATP as an indication of cell viability.

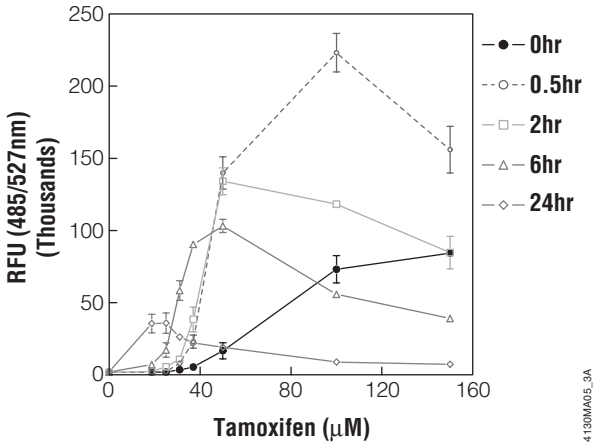


Figure 4. Characterization of the effects of tamoxifen on HepG2 cells using the Apo-ONE® Assay to measure caspase-3/7 activity as a marker of apoptosis.

The ATP data in Figure 3 indicate that high concentrations of tamoxifen are toxic after only one half-hour exposure. The longer the duration of tamoxifen exposure the lower the IC_{50} value or dose required to "kill" half of the cells, suggesting the occurrence of a cumulative cytotoxic effect. Both the concentration of toxin and the duration of exposure contribute to the cytotoxic effect. To illustrate the importance of taking measurements after an appropriate duration of exposure to test compound, notice that the ATP assay indicates that 30µM tamoxifen is not toxic at short incubation times but is 100% toxic after 24 hours of exposure. Choosing the appropriate incubation period will affect results.

The appearance of some apoptosis markers are transient and may only be detectable within a limited window of time. The data from the caspase assay in Figure 4 illustrate the transient nature of caspase activity in cells undergoing apoptosis. The total amount of caspase activity measured after 24 hours of exposure to tamoxifen is only a fraction of earlier time points. There is a similar trend of shifting to lower IC_{50} values after increased exposure time. The combined ATP and caspase data may suggest that, at early time points with intermediate concentrations of tamoxifen, the cells are undergoing apoptosis; but after 24 hours of exposure most of the population of cells are in a state of secondary necrosis.

Promega produces a complete portfolio of homogeneous assays that are designed to meet a variety of screening requirements.

Example Homogeneous Assays for Automated Screening

Promega produces a complete portfolio of homogeneous assays that are designed to meet a variety of screening requirements. The general protocol for these "homogeneous" assays is "add-mix-measure." Some of the assay systems require combining components to create the "Reagent," and some protocols require incubation or agitation steps, but none require removal of buffer or medium from assay wells. The available homogeneous assay systems (Table 1) include assays designed to measure cell viability, cytotoxicity and apoptosis.

MTS Cell Viability Assay

The CellTiter 96® AQ_{ueous} Assays are based on the reduction of the tetrazolium salt, MTS, to a colored formazan compound by viable cells in culture. The MTS tetrazolium is similar to the widely used MTT tetrazolium, with the advantage that the formazan product of MTS reduction is soluble in cell culture medium. Metabolism in viable cells produces "reducing equivalents" such as NADH or NADPH. These reducing compounds pass their electrons to an intermediate electron transfer reagent that can reduce MTS into the aqueous, formazan product. Upon cell death, cells rapidly lose the ability to reduce tetrazolium products. The production of the colored formazan product, therefore, is proportional to the number of viable cells in culture.

The CellTiter 96® AQ_{ueous} One Solution Cell Proliferation Assay[a] is an MTS-based assay that involves adding a reagent directly to the assay wells at a recommended ratio of 20µl reagent to 100µl of culture medium. Cells are incubated 1–4 hours at 37°C, and then absorbance is measured at 490nm. Figure 5 provides an example of an experiment to determine the IC_{50} value of tamoxifen using the CellTiter 96® AQ_{ueous} One

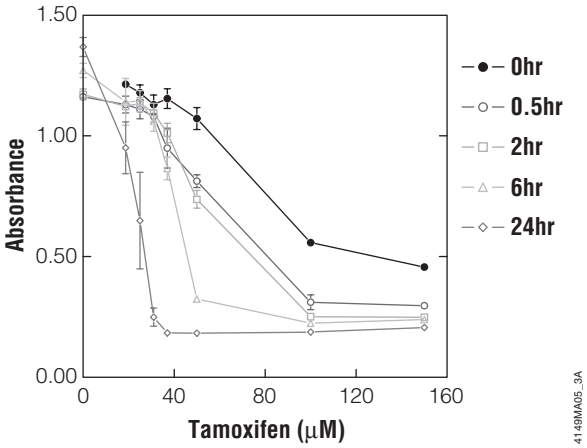


Figure 5. Characterization of the toxic effects of tamoxifen on HepG2 cells using the CellTiter 96® AQ_{ueous} Assay to measure MTS reduction as an indication of cell viability.

Solution Cell Proliferation Assay. The detection sensitivity is approximately 1,000 cells. This assay chemistry has been widely accepted and is cited in hundreds of published articles.

ATP Assay of Cell Viability

The amount of ATP in cells correlates with cell viability. Within minutes after a loss of membrane integrity, cells lose the ability to synthesize ATP, and endogenous ATPases destroy any remaining ATP, thus the levels of ATP fall precipitously and rapidly.

The CellTiter-Glo® Luminescent Cell Viability Assay[b] is a homogeneous method to determine the number of viable cells in culture. Detection is based on using the luciferase reaction to measure the amount of ATP in viable cells. The CellTiter-Glo® Reagent does three things upon addition to cells. It lyses cell membranes to release ATP, it inhibits endogenous ATPases, and it provides luciferin and luciferase necessary to measure ATP using a bioluminescent reaction. The unique properties of a proprietary stable luciferase mutant enabled development of a robust, single-addition reagent. The "glow-type" signal can be recorded with a luminometer, CCD camera or modified fluorometer and generally has a half-life of five hours, providing a consistent signal across large batches of plates.

The CellTiter-Glo® Assay is extremely sensitive and can detect as few as 15 cells. Although equilibration of assay plates to room temperature is recommended before the assay for consistency, the assay can be done rapidly. The luminescent signal can be detected as soon as 10 minutes after adding reagent or several hours later, providing flexibility for batch processing of plates. Example data are shown in Figure 3.

Resazurin Reduction Cell Viability Assay

Resazurin can enter living cells where it is reduced to the fluorescent resorufin product. The conversion of resazurin to resorufin is proportional to the number of metabolically active, viable cells present in a population.

The CellTiter-Blue™ Cell Viability Assay uses an optimized reagent containing resazurin. The homogeneous procedure involves adding the reagent directly to cells in culture at a recommended ratio of 20µl of reagent to 100µl of culture medium. The assay plates are incubated at 37°C for 1–4 hours to allow viable cells to convert resazurin to the fluorescent resorufin product. The signal is recorded using a standard multiwell fluorometer. Because different cell types have different abilities to reduce resazurin, optimizing the length of incubation with the CellTiter-Blue™ Reagent can be used to improve assay sensitivity for a given model system. The detection sensitivity is intermediate between the ATP assay and the MTS reduction assay (Table 1).

The CellTiter-Blue™ Assay is a simple, inexpensive procedure that has the potential for being multiplexed with other assays to collect a variety of data (Figure 6). The incubation period of the reagent with the cells is flexible, and the data can be collected using either fluorescence or absorbance. The assay provides good Z´-factor values in high-throughput screening situations as well (see Technical Bulletin #TB317).

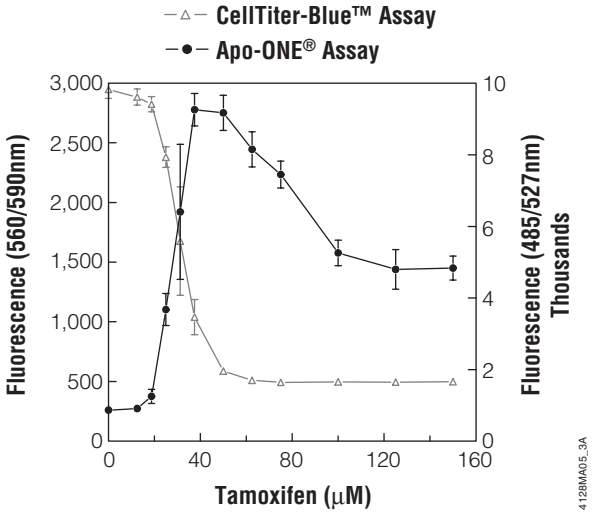


Figure 6. Multiplexing cell viability assays. HepG2 cells (10,000 cells/100µl cultured overnight) were treated with various concentrations of tamoxifen for 5 hours. Viability was determined by adding CellTiter-Blue™ Reagent (20µl/well) to each well after 3.5 hours of drug treatment and incubating for 1 hour prior to recording fluorescence ($560_{Ex}/590_{Em}$). Caspase activity was then determined by adding 120µl/well of Apo-ONE® Homogeneous Caspase-3/7 Reagent and incubating for 0.5 hour prior to recording fluorescence ($485_{Ex}/527_{Em}$).

LDH-Release Cytotoxicity Assay

Cells that have lost membrane integrity release lactate dehydrogenase (LDH) into the surrounding medium. The CytoTox-ONE™ Homogeneous Membrane Integrity Assay[b] is a fluorescent method that uses coupled enzymatic reactions to measure the release of LDH from damaged cells as an indicator of cytotoxicity. The assay is designed to estimate the number of nonviable cells present in a mixed population of living and dead cells. Alternatively, if cell lysis reagent is used, the same assay chemistry can be used to determine the total number of cells in a population.

LDH catalyzes the conversion of lactate to pyruvate with the concomitant production of NADH. The CytoTox-ONE™ Reagent contains excess substrates (lactate and NAD+) to drive the LDH reaction and produce NADH. This NADH, in the presence of diaphorase and resazurin, is used to drive the diaphorase-catalyzed production of the fluorescent resorufin product. By developing reaction conditions that proceed at physiological pH and salt conditions, the CytoTox-ONE™ Reagent does not damage living cells, and the assay can be performed directly in cell culture using a homogeneous method. The CytoTox-ONE™ Assay is fast, typically requiring only a 10-minute incubation period. Under these assay conditions, there is not a significant reduction of resazurin by the population of viable cells.

The major advantage of the Apo-ONE® Assay is that the profluorescent R110 substrate allows more sensitive detection than other fluorescent caspase assays.

The CytoTox-ONE™ Assay is compatible with 96- and 384-well formats. The detection sensitivity is a few hundred cells (Table 1) but can be limited by the LDH activity present in serum used to supplement culture medium. When automated on the Biomek® 2000 workstation, the CytoTox-ONE™ Assay gave excellent Z´-factor values (Figure 7).

Fluorescent Caspase-3/7 Assay to Detect Apoptosis

Caspases are proteolytic enzymes that become activated during the process of apoptosis and cleave a variety of target proteins. The activation of caspases leads to the destruction of integral intracellular DNA repair elements, structural polypeptides and signaling kinases. Detection of the activity of executioner caspases such as caspase-3 and -7 is a reliable indicator of apoptosis in cells.

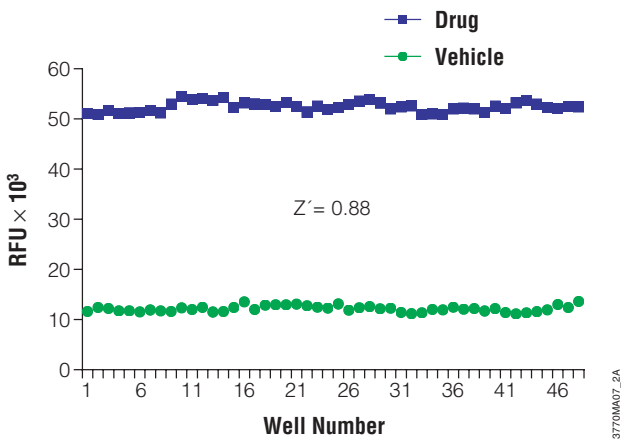


Figure 7. Representation of Z´-factor equal to 0.88 using the CytoTox-ONE™ Assay for one of the plates processed by the Biomek® 2000 workstation. HepG2 cells were plated in 96-well tissue culture-treated white plates with clear bottoms at a density of 40,000 cells/well. Cells were allowed to grow to confluence and then treated with either 3.125µM staurosporine on one half of the plate or the DMSO vehicle control on the other half. A two-plate protocol was written for the Biomek® 2000 workstation, and plates were read on a fluorescent plate reader.

The Apo-ONE® Homogeneous Caspase-3/7 Assay[c] detects caspase-3/7 activity based on the cleavage of a profluorescent DEVD peptide-rhodamine 110 substrate [(Z-DEVD)2-R110]. The Apo-ONE® Reagent is prepared by combining buffer and substrate. The reagent is added directly to culture wells using a 1:1 ratio of reagent to culture medium. The contents of the well are mixed and incubated at ambient temperature, typically for 1–2 hours, and the fluorescent signal is measured using a fluorometer. The reagent permeabilizes the cells to release the caspase, delivers the profluorescent substrate, and provides optimized conditions to stabilize caspase activity. Because the fluorescent R110 product continues to accumulate in the presence of active caspase-3 and -7, extending the incubation period up to 18 hours increases the signal-to-background ratio, providing greater sensitivity. The Apo-ONE® Assay is easily scalable to meet the miniaturization needs of high-throughput screening as long as the 1:1 ratio of reagent:sample is maintained.

The major advantage of the Apo-ONE® Assay is that the profluorescent R110 substrate allows more sensitive detection than other fluorescent caspase assays. R110 has a higher extinction coefficient, greater fluorescence quantum yield, and is more photostable than the coumarins (AMC and AFC) commonly used in other caspase assays. The detection sensitivity is in the range of several hundreds of cells (Table 1) but can be influenced by the length of incubation. An example set of data from the Apo-ONE® Assay is shown in Figure 6.

Luminescent Caspase-3/7 Assay to Detect Apoptosis

The Caspase-Glo™ 3/7 Assay[b] is the newest addition to the portfolio of homogeneous assays. It measures caspase-3/7 activity based on the cleavage of a DEVD peptide-amino-luciferin substrate. Caspase cleavage of the substrate liberates free aminoluciferin, which can be used as a substrate by luciferase to generate light.

The Caspase-Glo™ Reagent is made by combining a lyophilized substrate and buffer. The reagent is added directly to cells in culture at a 1:1 ratio of reagent to culture medium. The cells and reagent are mixed and incubated at ambient temperature for 30 minutes to 3 hours, and luminescence is recorded. The assay has a flexible incubation time for recording the "glow-type" luminescent signal. When steady state is reached after approximately 30 minutes to one hour of incubation, the luminescent signal of this coupled enzymatic assay is directly proportional to the amount of caspase-3 and -7 activity over a broad linear range (see article on page 13 of this issue).

The Caspase-Glo™ Assay is the most sensitive caspase assay available. The level of detection sensitivity is approximately 20 apoptotic cells (Table 1, TB323). This luminescent assay is so sensitive that it can detect caspase activity in serum. Because the assay measures luminescence, it has the added advantage that fluorescent compounds will not interfere with results.

Additional Factors to Consider When Choosing an Assay

Among the many factors to consider when choosing a cell-based assay, the primary concern for many researchers is **ease of use**. Homogeneous assays do not require removal of culture medium, cell washes or centrifugation steps. When choosing an assay, the time required for reagent preparation and the total length of time necessary to develop a signal from the assay chemistry should be considered. The stability of the absorbance, fluorescence, or luminescence signal is another important factor that provides convenience and flexibility in recording data and minimizes differences when processing large batches of plates.

Sensitivity of detection is another factor to consider when selecting an assay. Detection sensitivity will vary with cell type if you choose to measure a metabolic marker, such as ATP levels or MTS tetrazolium reduction. The sensitivity of some assays may improve by increasing signal-to-background ratios with increasing time of incubation. The sensitivity depends upon the parameter measured but also on other characteristics of the model system such as the plate format and number of cells used per well. Cytotoxicity assays that are designed to detect a change in viability in a population of 10,000 cells may not require the most sensitive assay technology. For example, a tetrazolium assay should easily detect the difference between 10,000 and 8,000 viable cells. On the other hand, assay model systems that use low cell numbers in a high-density multiwell plate format may require maximum sensitivity of detection above background such as the luminescent ATP assay technology.

For researchers using automated screening systems, the **reagent stability** and compatibility with robotic components is often a concern. The assay reagents must be stable at ambient temperature for an adequate period of time. In addition, the stability of the signal generated by the assay should also be stable for extended periods of time to allow flexibility for recording data. For example, the luminescent signal from the ATP assay has a half-life of about 5 hours providing adequate flexibility for recording data. With other formats such as the MTS tetrazolium assay or the LDH release assay, the signal can be stabilized by the addition of a detergent containing Stop Solution.

> The Caspase-Glo™ Assay is the most sensitive caspase assay available.

In some cases the choice of assay may be dictated by the **availability of instrumentation** to detect absorbance, fluorescence, or luminescence. The Promega portfolio of products contains an optional detection format for each of the three major classes of cell-based assays (viability, cytotoxicity, or apoptosis). In addition, some assays such as the ATP assay can be recorded with more than one type of instrument (luminometer, fluorometer, or CCD camera).

Cost is an important consideration for every researcher; however, many things that factor into the total cost of running an assay are often overlooked. All of the assays described above are homogeneous and as such are more efficient than multistep assays. For example, the speed (time savings), sensitivity (cell sample savings), and accuracy of an ATP-based assay may outweigh the initial cost. Assays with good detection sensitivity that are easier to scale down to 384- or 1536-well formats may result in savings of cell culture reagents and enable testing of very small quantities of expensive or rare test compounds.

The ability to gather more than one set of data from the same sample (i.e., **multiplexing**) also may contribute to saving time and effort. Multiplexing more than one assay from the same culture well can provide internal controls and eliminate the need to repeat work. Figure 6 shows an example of multiplexing a cell viability assay (CellTiter-Blue™ Assay) and a caspase assay (Apo-ONE® Assay) sequentially in the same well. The LDH-release assay is another example of an assay

that can be multiplexed. The LDH-release assay offers the opportunity to gather cytotoxicity data from small aliquots of culture supernatant that can be removed to a separate assay plate, thus leaving the original assay plate available for any other assay including gene reporter, image analysis, etc.

The **reproducibility of data** is an important consideration when choosing a commercial assay. However, for most cell-based assays, the variation among replicate samples is more likely to be caused by the cells rather than the assay chemistry. Variations during plating of cells can be magnified by using cell lines that tend to form clumps rather than a suspension of individual cells. Extended incubation periods and edge effects in plates may also lead to decreased reproducibility among replicates and increased Z´-factor values.

An often overlooked aspect in choosing an assay is **technical support.** Promega Technical Services department includes a staff of trained scientists available by telephone, e-mail or fax to answer your questions or help with troubleshooting. Additional detailed technical information about assay chemistry and automated methods is available online from the Promega web site.

Summary

Choosing the right cell-based assay and endpoint is important for generating high-quality data that answer your research questions with the greatest accuracy and speed. The Promega cell-based assay portfolio provides a wide range of assays suitable for a variety of high-throughput screening needs. These products are backed up by Promega's commitment to quality, reliability, and technical support. For further information about Promega cell-based assays, visit our web site at www.promega.com/techserv. ■

Protocols

CellTiter 96® AQ_{ueous} One Solution Cell Proliferation Assay Technical Bulletin #TB245
(www.promega.com/tbs/tb245/tb245.html)

CellTiter-Glo® Luminescent Cell Viability Assay Technical Bulletin #TB288
(www.promega.com/tbs/tb288/tb288.html)

CellTiter-Blue™ Cell Viability Assay Technical Bulletin #TB317
(www.promega.com/tbs/tb317/tb317.html)

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

Apo-ONE® Homogeneous Caspase-3/7 Assay Technical Bulletin #TB295
(www.promega.com/tbs/tb295/tb295.html)

Caspase-Glo™ 3/7 Assay Technical Bulletin #TB323
(www.promega.com/tbs/tb323/tb323.html)

Ordering Information

Cell Viability Assays

Product	Size	Cat.#
CellTiter-Glo® Luminescent Cell Viability Assay[b]	10ml	G7570
	10 × 10ml	G7571
	100ml	G7572
	10 × 100ml	G7573
CellTiter 96® AQ_{ueous} One Solution Cell Proliferation Assay[a]*	200 assays	G3582
	1,000 assays	G3580
	5,000 assays	G3581
CellTiter-Blue™ Cell Viability Assay	20ml	G8080
	100ml	G8081
	10 × 100ml	G8082

*For Laboratory Use.

Cytotoxicity and Apoptosis Assays

Product	Size	Cat.#
CytoTox-ONE™ Homogeneous Membrane Integrity Assay[b]	200–800 assays	G7890
	1,000–4,000 assays	G7891
	1,000–4,000 assays (bulk)	G7892
Apo-ONE® Homogeneous Caspase-3/7 Assay[c]	1ml	G7792
	10ml	G7790
	100ml	G7791
Caspase-Glo™ 3/7 Assay[b]	2.5ml	G8090
	10ml	G8091
	100ml	G8092

[a]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.

[b]Patent Pending.

[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.