ApoTox-Glo™ Triplex Assay

Assay for live, dead and apoptotic cells in the same well

- ✓ Sensitive, non-lytic, fluorescent assays quantitate live and dead cells
- ✓ Sensitive luminescent assay for caspase 3/7 activation.
- ✓ Simple two-step add-mix-measure protocols
- ✓ Adaptable to 96-, 384- & 1,536-well plate assays

Fisher Scientific

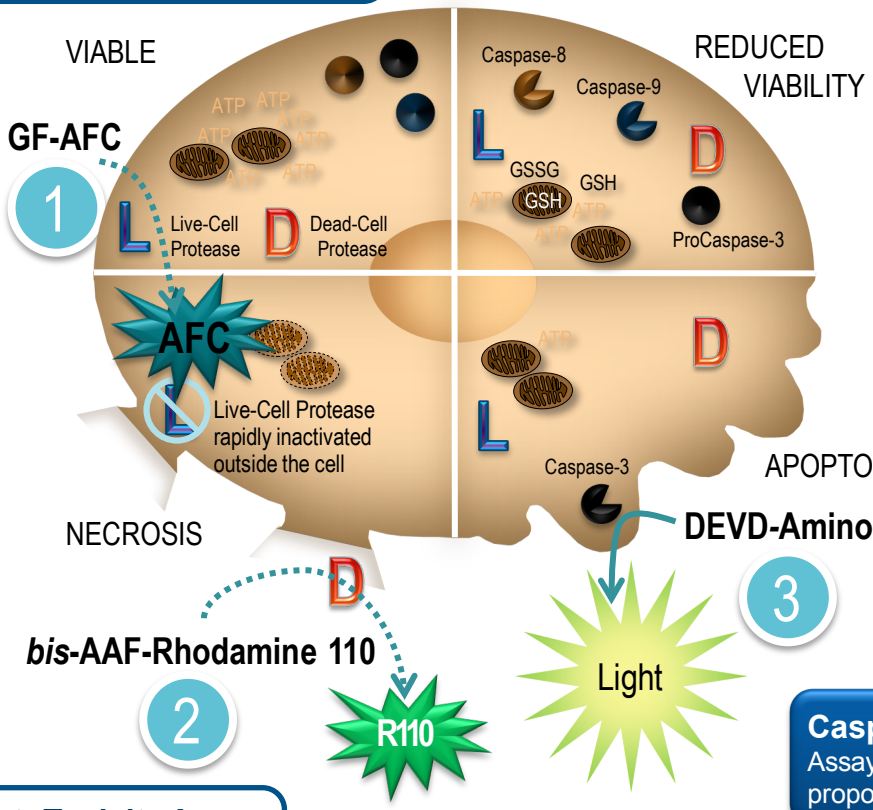
Promega

Triplex Principles

Live/Dead Cells
+ Apoptotic Cells

Cell Death Mechanism

Viability Assay
Reaction occurs within living cells. AFC fluorescence is proportional to live cells



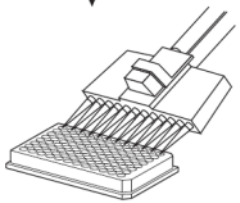
CytoToxicity Assay
Reaction occurs in the medium. R110 fluorescence proportional to dead cells..

Caspase-Glo® 3/7 Assay
Assay lyses cells. Luminescence is proportional to caspase-3/7 activity.

Protocol Overview

Go to the ApoTox-Glo™ Triplex Assay Technical Manual

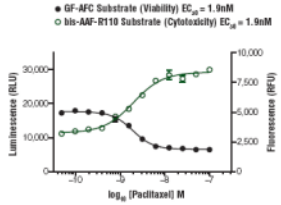
Treat cells grown in black or white plates with compound of interest (typically, 0-24 hours)



Add Cytotoxicity/Viability Reagent. Mix & incubate 30 minutes

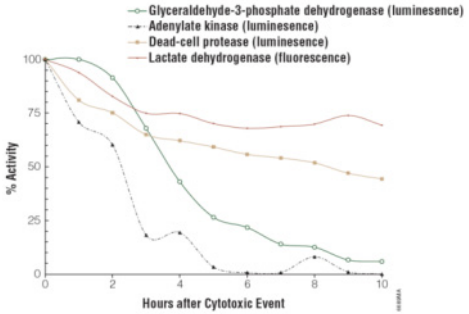
Measure
AFC fluorescence
380-400nm$_{Ex}$/505nm$_{Em}$
R110 fluorescence
485nm$_{Ex}$/520nm$_{Em}$

Measure fluorescence.

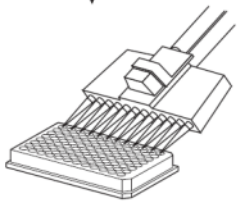


Ratiometric Live/Dead Cells

Timing of assays to measure a released cellular enzyme is critical. You must consider the half-life of the enzyme outside the cell.



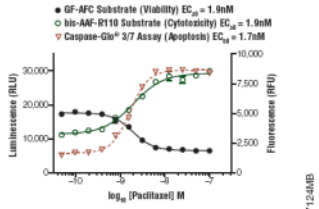
Half-life of enzymatic markers for cytotoxicity



Add Caspase-Glo® 3/7 Reagent. Mix & incubate 30 minutes

Measure
Luminescence

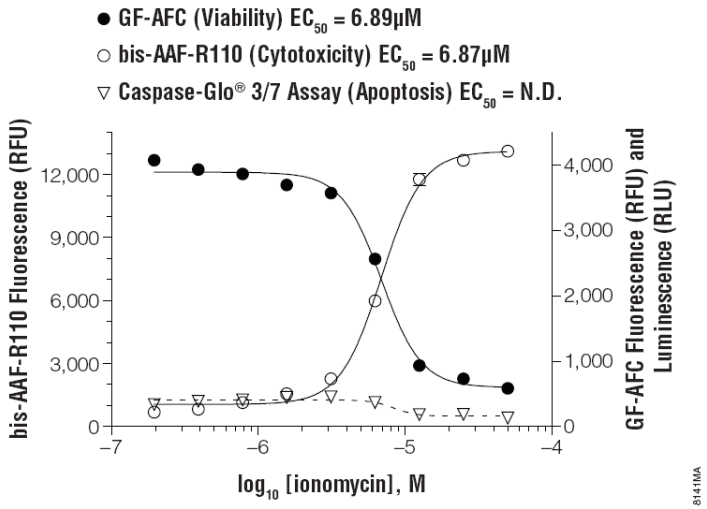
Measure luminescence.



Live, Dead and Apoptotic Cells

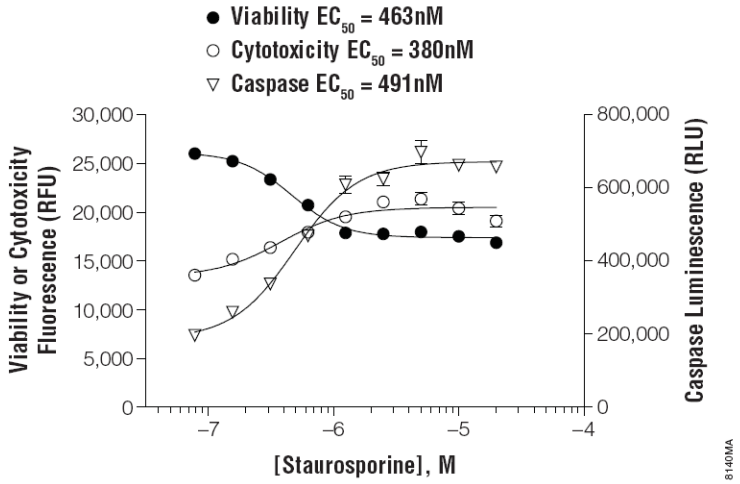
7124MB

Necrosis or Apoptosis defined



Necrotic Cell Death

Little or no caspase-3/7 activation



Apoptotic Cell Death

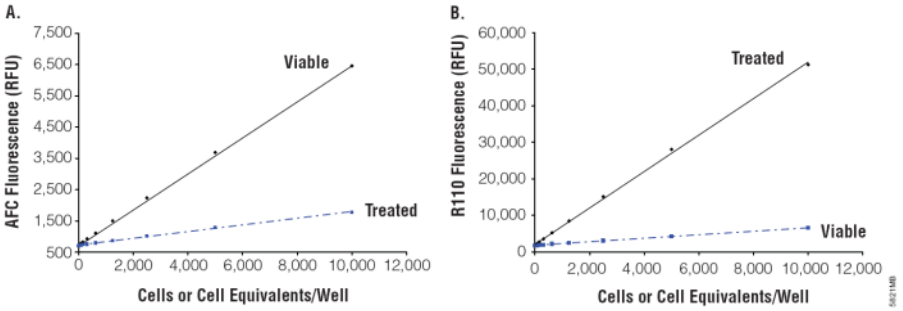
Caspase-3/7 activation

Jurkat cells treated for 6 hours with either ionomycin or staurosporine

Sensitivity

Viability Assay
Live Cell Detection Limits
96-well: ~400 cells
384-well:~50 cells

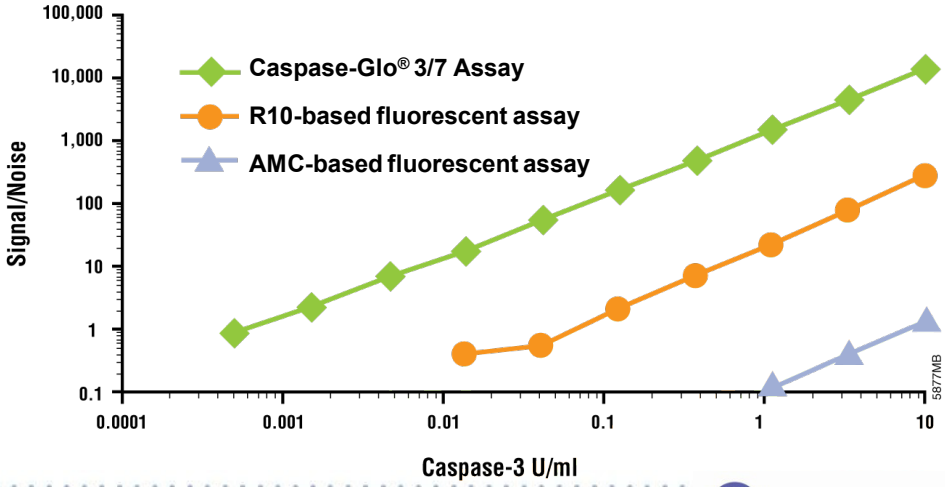
Cytotoxicity Assay
Dead Cell Detection
Limits 2-5% dead cells
per well



A pool of Jurkat cells was divided into two fractions. One fraction was treated to simulate cytotoxicity, whereas the other was left untreated.

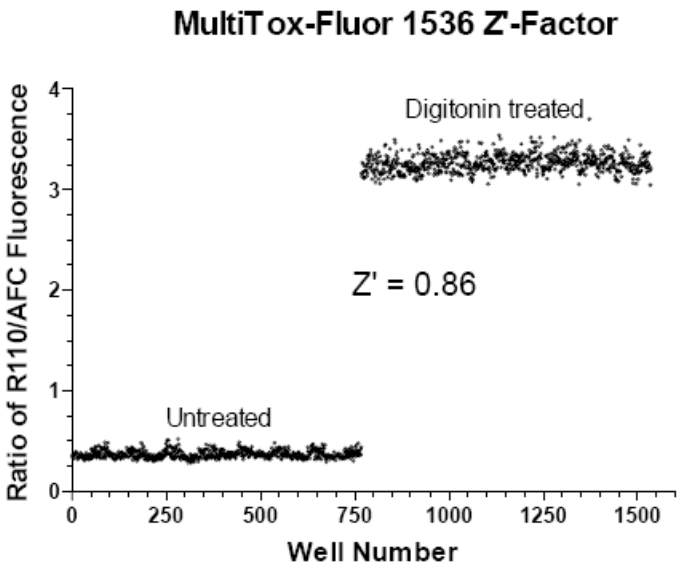
Detect caspase-3/7 activation earlier and with fewer cells.

The Caspase-Glo® 3/7 Assay can detect caspase 3/7 activation at lower levels than fluorescent methods.



Flexibility for High-Throughput Assays

The ApoTox-Glo Assay combines the MultiTox-Fluor and Caspase-Glo® 3/7 Assays giving tight CVs and excellent Z' values.



Full plates of Jurkat cells (2,500/well 1,536-well plates) were prepared. Half of each plate was left untreated and the other half got digitonin . The cells were assayed simultaneously for dead cells (CytoTox-Fluor™ Assay; AAF-Rhodamine 110 substrate) and live cells (CellTiter-Fluor™ Assay; GF-AFC substrate).

Source: Niles, A., Worzella, T. and Busch, M. (2008) Automation of multiplexed cell-based viability and cytotoxicity assays. *Presented at the Society for Biomolecular Sciences (SBS).*

Caspase-Glo® 3/7 Assay

Format	Assay Volume	Cells/ Well	%CV[1]	Z' Factor[2]
1536	8µl	2000	5.27	0.77
	5µl	1250	5.07	0.79
	2µl	500	7.78	0.71
	1µl	250	6.12	0.62
	0.5µl	125	8.53	n.t.

[1] The %CV data was obtained by plating a serial dilution of Jurkat or d293 cells and inducing apoptosis with either anti-FAS antibody or Triton®-X 100. The Caspase-Glo 3/7 Reagent was added and light units recorded. The %CV values listed here were calculated from the maximum cell number used in each titration series.

[2] The Caspase-Glo 3/7 Z'-factor assays were performed by plating Jurkat cells and treating one-half of the plate with an anti-FAS antibody for four hours, with the remaining half receiving no treatment. The Caspase-Glo 3/7 Reagent was then added and light units were recorded.

Source: Worzella, T., et al. (2006) Automating Promega CellTiter-Glo® Luminescent Cell Viability and Caspase-Glo® 3/7 Assays in Low-Volume 384 and 1536-Well Formats. *Society for Biomolecular Sciences (SBS)* meeting poster.

Ordering Information

Product	Size	Catalog Number
ApoTox-Glo™ Triplex Assay[a,b,c]	10ml	PRG6320
	5 x 10ml	PRG6321

For Laboratory Use. G6320 contains sufficient reagents for 100 assays at 100µl/assay in a 96-well plate format or 400 assays at 25µl/assay in a 384-well format. G6321 contains sufficient reagents for 500 assays at 100µl/assay in a 96-well plate format or 2,000 assays at 25µl/assay in a 384-well format.

Fisher Scientific

For customer service, call 1-800-766-7000.
To fax an order, use 1-800-926-1166.
To order online: www.fishersci.com

Questions?

e-mail: Technical Services
techserv@promega.com

800-356-9526, Option 4
Available 8am-7pm Eastern
Monday-Friday

Links to more information

ApoTox-Glo™ Assay

Shultz, S., *et al.* (2009) Automated triplex assay to assess cell viability, cytotoxicity and apoptosis. *Presented at Lab Automation 2009 Conference.*

MultiTox-Fluor and/or Caspase-Glo® 3/7 Assay

Niles, A., *et al.* (2006) Multiplexed viability, cytotoxicity and apoptosis assays for cell-based screening. *Cell Notes* **16**, 12-15.

Niles, A., *et al.* (2008) Using protease biomarkers to measure viability and cytotoxicity. *Cell Notes* **19**, 16-20.

Zakowicz, H., *et al.* (2008) Measuring cell health and viability sequentially by same-well multiplexing using the GloMax®-Multi Detection System. *Promega Notes* **99**, 25-28.

Worzella, T., Busch, M. and Niles, A. (2008) High-throughput automation of multiplexed cell-based assays for viability and cytotoxicity. *Cell Notes* **20**, 26-29.

Niles, A.L., *et al.* (2006) MultiTox-Fluor Multiplex Cytotoxicity Technology. *Cell Notes* **15**, 11-15.

Niles, A., *et al.* (2007) Measure relative numbers of live and dead cells and normalize assay data to cell number. *Cell Notes* **18**, 15-20.

He, J.-Q., Ma, J. and Anson, B. (2009) Use of pluripotent stem cell-derived cardiomyocytes to understand mechanisms of cardiotoxic compounds. *Cell Notes* **23**, 10-13.

O'Brien, M., Moravec, R. and Riss, T. (2003) Caspase-Glo 3/7 Assay: Use fewer cells and spend less time with this homogeneous assay. *Cell Notes* **6**, 13-15.

MultiTox-Fluor HighWire Press®

MultiTox-Fluor from Nature.com

MultiTox-Fluor PubChem BioAssays

Caspase-Glo 3/7 HighWire Press®

Caspase-Glo 3/7 from Nature.com

Caspase-Glo 3/7 PubChem BioAssays