MultiTox-Fluor Multiplex Cytotoxicity Assay

One addition assay for both viability and cytotoxicity in the same well

- ✓ *Normalize data to reduce false positives and negatives*
- ✓ *Sensitive, non-lytic assays*
- ✓ *Simple add-mix-measure protocol*
- ✓ *Flexible, multiplex-friendly format*

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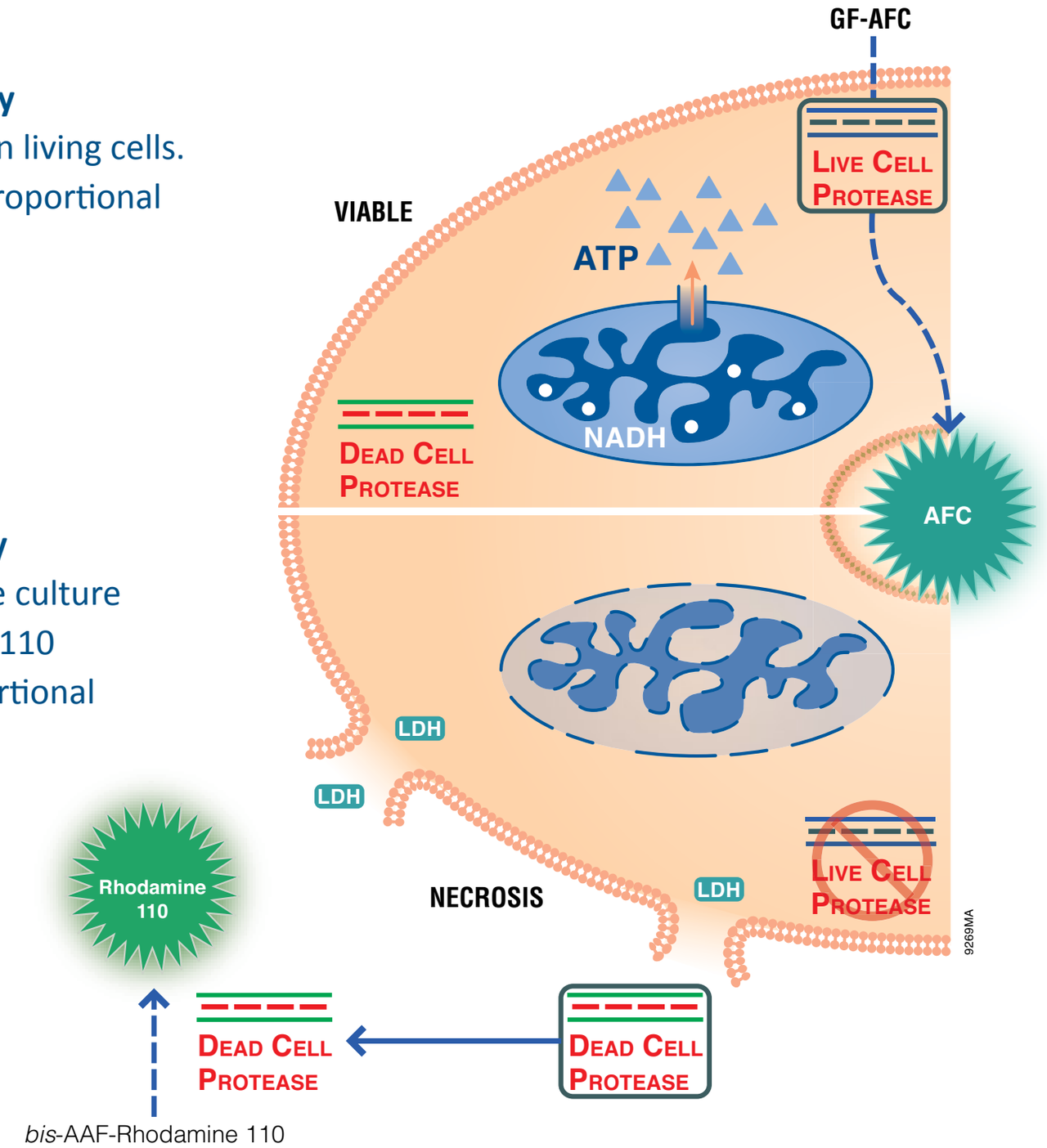
Principle

MultiTox-Fluor gives ratiometric live cell to dead cell data

CellTiter-Fluor™ Assay
Reaction occurs within living cells. AFC fluorescence is proportional to live cells

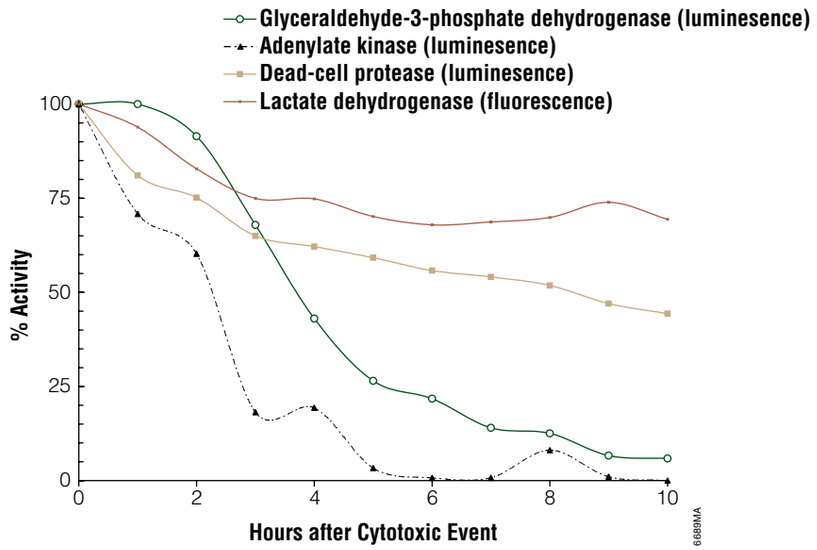
CytoTox-Fluor™ Assay
Reaction occurs in the culture medium. Rhodamine 110 fluorescence is proportional to dead cells.



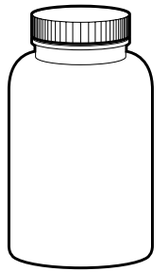
Protocol Overview

MultiTox-Fluor Technical Bulletin

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

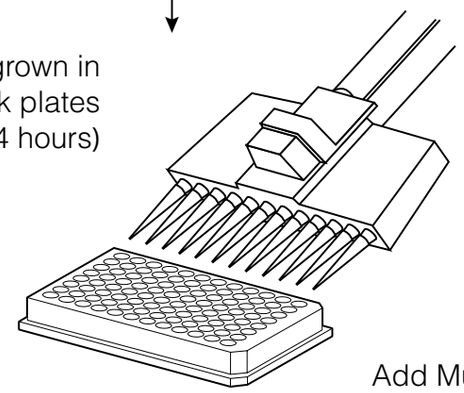


Half-life of enzymatic markers for cytotoxicity



MultiTox-Fluor Multiplex Cytotoxicity Assay Reagent

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



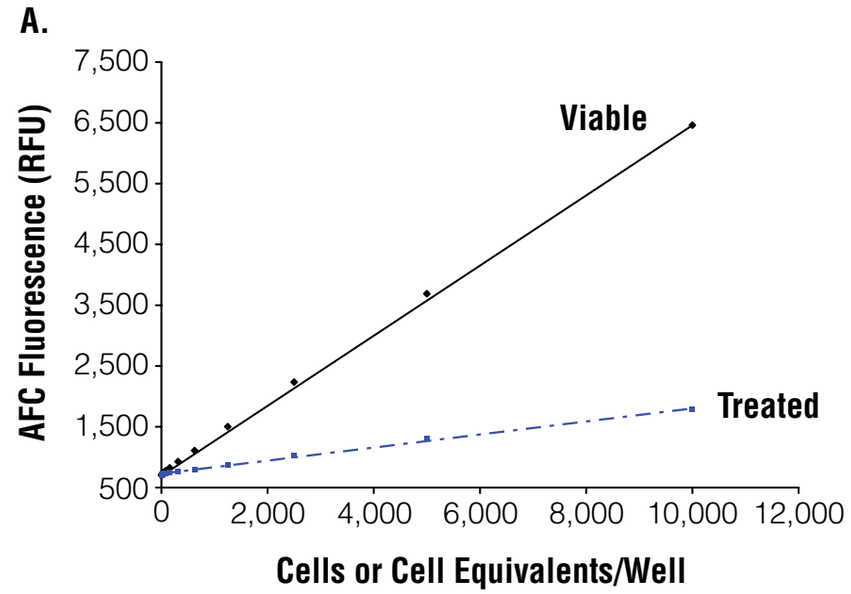
Add MultiTox Reagent
Mix & incubate 30 minutes

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

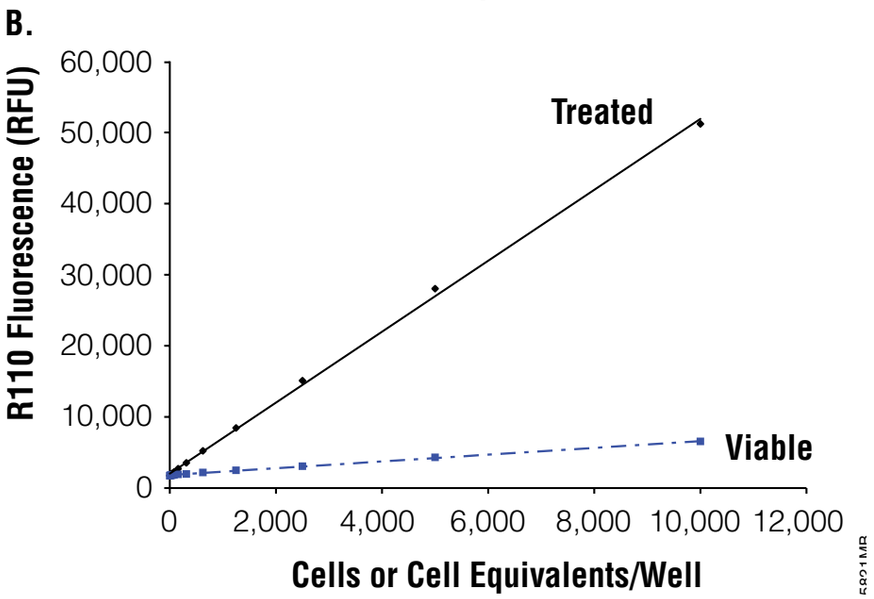
GloMax® -Multi + Detection System
or GloMax-Multi Detection System

9270TA

Sensitivity



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



MultiTox-Fluor 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.

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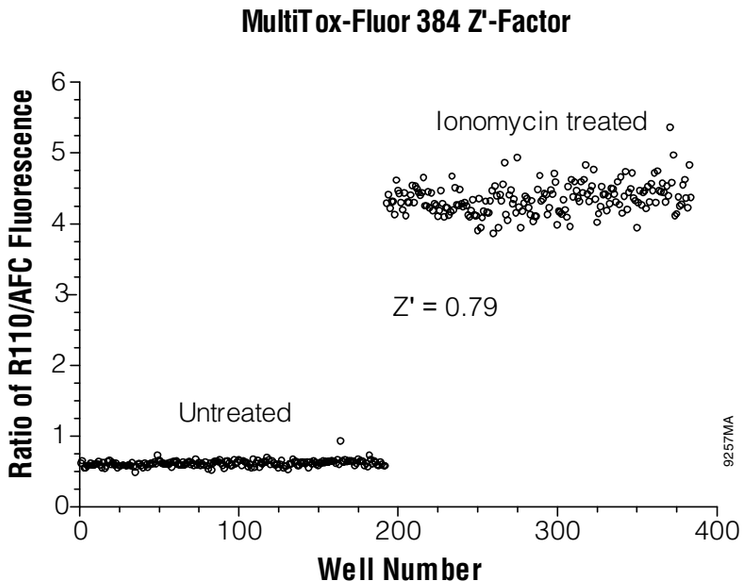
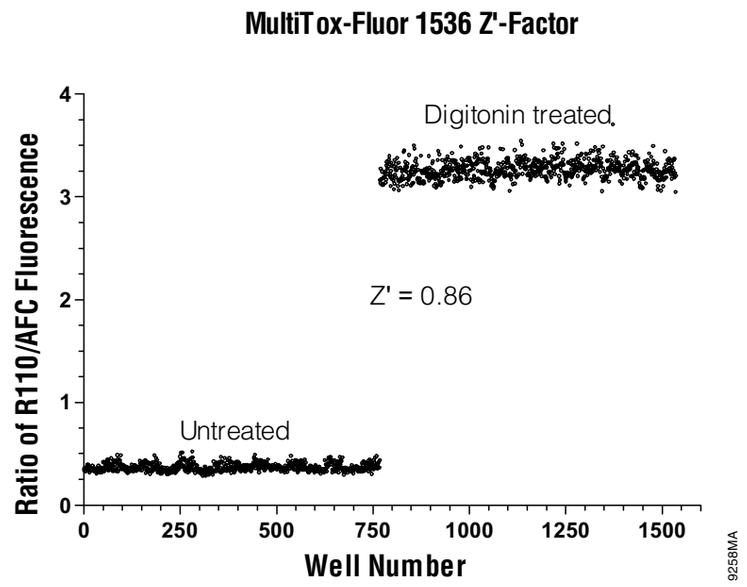
Flexibility for High Throughput Assays

Excellent Z'-Factor Values for 384- & 1,536-well assays





Full plates of Jurkat cells (5,000/well 384-well plates; 2,500/well 1,536-well plates) were prepared. Half of each plate was left untreated and the other half got either ionomycin (384-well) or digitonin (1,536-well). 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).

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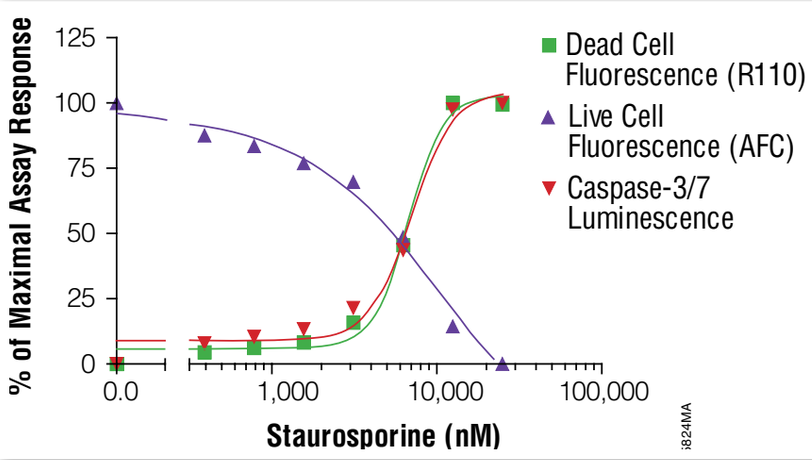
Triplexing for even more data

MultiTox-Fluor Assay is easy to incorporate into your workflow

ASSAY	CAT.#	COMMENTS
Caspase-Glo® 3/7 Assay	G8091	Understand viability/cytotoxicity as it relates to progression into apoptosis. Read live cel fluorescence then dead cell fluoresence followed by the luminescent caspase assay. MultiTox-Fluor and Caspase-Glo 3/7 Assays are together as the **ApoTox-Glo™ Triplex Assay** (Cat. #G6320)
Caspase-Glo 8 Assay	G8201	
Caspase-Glo 9 Assay	G8211	
GSH-Glo™ Glutathione Assay	V6912	Understand changes in viability /cytotoxicity as it relates to changes in glutathione level under conditions of oxidative stress. Read live cel fluorescence then dead cell fluoresence followed by the luminescent glutathione assay.
Bright-Glo™ Luciferase Assay System	E2620	Understand gene expression in the context of live/dead cells Read live cell fluorescence then dead cell fluoresence followed by the luciferase assay.
Steady-Glo® Luciferase Assay System	E2520	
ONE-Glo™ Luciferase Assay System	E6120	



Apoptosis confirmed as mechanism of cytotoxicity

LN-18 cells (10,000/well; 96-well plate) were treated for 6 hours. MultiTox-Fluor was added and incubated for 30 minutes. Caspase-Glo 3/7 Assay was incubated for 10 minutes.

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Ordering Information

PRODUCT	SIZE	CATALOG NUMBER
MultiTox-Fluor Multiplex Cytotoxicity Assay[a]	10ml	G9200
	5 x 10ml	G9201
	2 x 50ml	G9202

For Laboratory Use. G9200 contains sufficient reagents for 100 assays at 100µl/assay in a 96-well format or 400 assays at 25µl/assay in a 384-well format. G9201 contains sufficient reagents for 500 assays at 100µl/assay in a 96-well format or 2,000 assays at 25µl/assay in a 384-well format. G9202 contains sufficient reagents for 1,000 assays at 100µl/assay in a 96-well format or 4,000 assays at 25µl/assay in a 384-well format.

[a]Patent Pending.

Resources

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

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

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.

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

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.

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.

Citations from HighWire Press®

Assays in PubChem BioAssays

ApoTox reference from PubHub by Sarah Shultz

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