

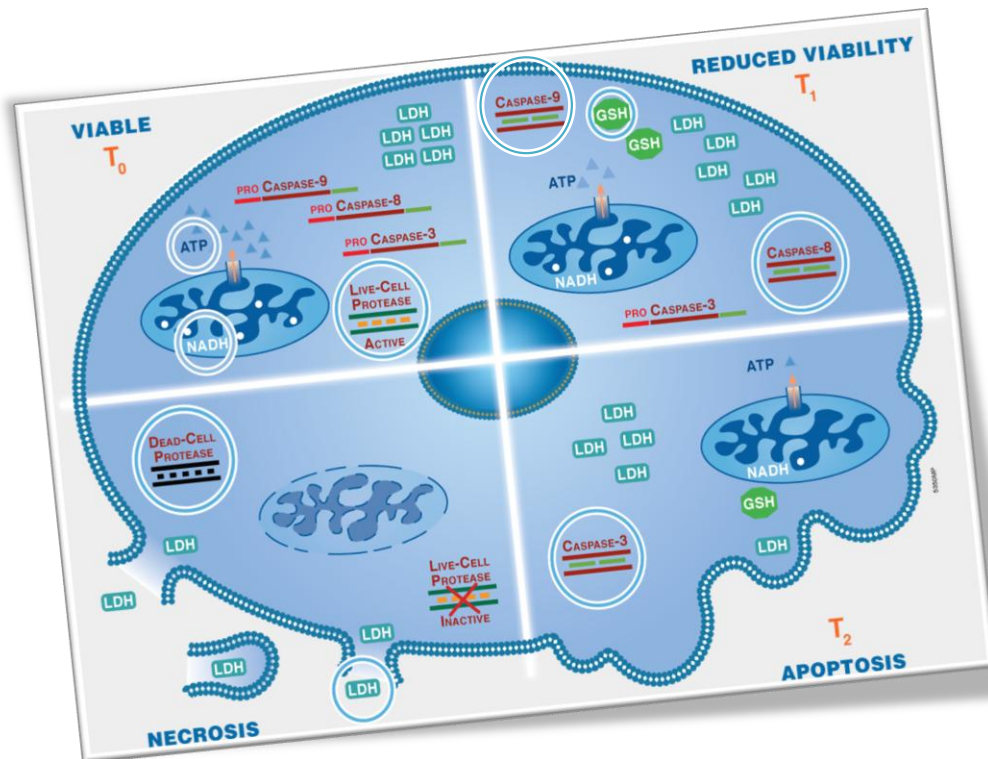
Multiplexing Cell-Based Assays: Get More Biologically Relevant Data

Fall 2010



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speakers notes for
each slide.

Multiplexing assays for more informative data

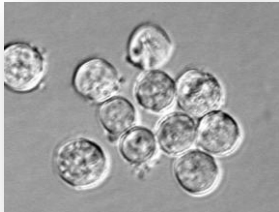


- Plate-based assays for viability, cytotoxicity and apoptosis measurement
- Using multiplex assays to understand cell death mechanism
- Monitoring cell response in multiple applications



Definitions

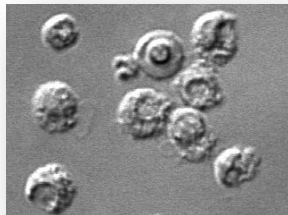
Cell Viability Assay



viable cells

- Assays based on measuring a cytoplasmic enzyme or marker in cells with intact cell membranes. Assays could be lytic or non-lytic.

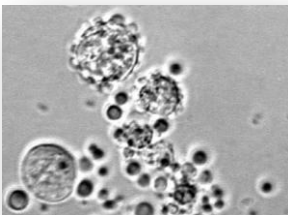
Cytotoxicity Assay



1° necrosis

- Assays based on cells that do not have intact cell membranes leaking normally cytoplasmic enzymes into the cell culture medium. Assays are non-lytic.

Apoptosis Assay



apoptosis with 2° necrosis

- Assays based on measuring activation of specific caspases. Cells with activated caspase-3/7 are considered committed to apoptotic cell death. Assays are lytic.



Multiplexing is...



Gathering more than one set of data from the same sample

Assays must be chemically & biologically compatible

- Signals must be spectrally distinct
- Assay chemistries must be compatible
- The two assays must fit in the available volume of the well or be separable.

Assay #1

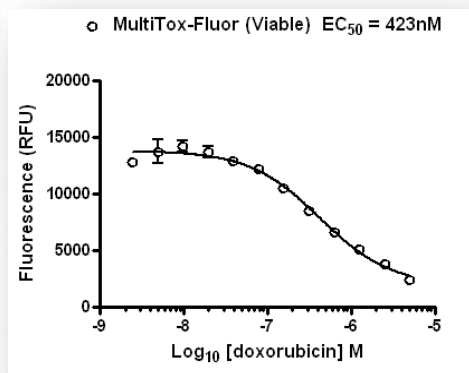
Assay #2



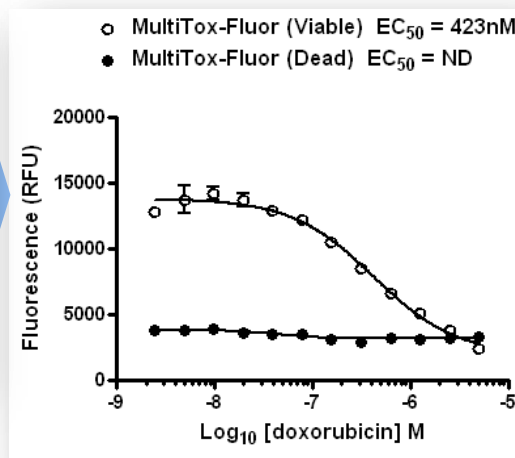


What is the motivation for multiplexing?

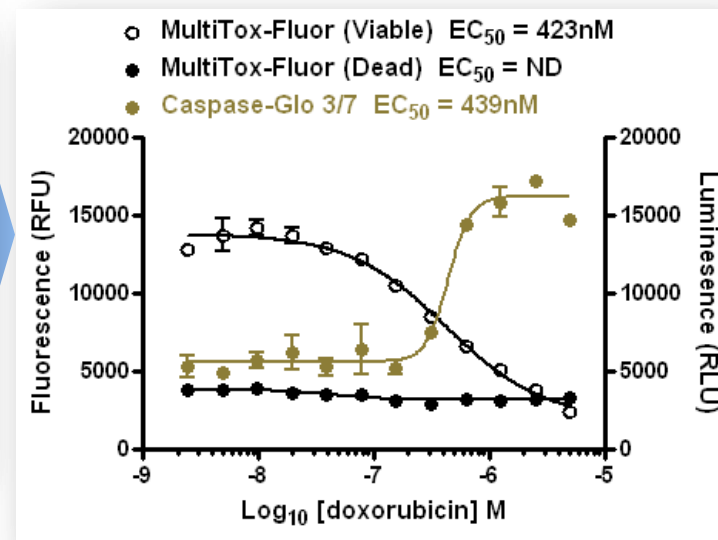
A more complete picture of what is happening to the cell.



Cells are apparently losing viability...



...but the membranes are intact.
How?



Apparent loss of viability, intact membranes, caspase-3/7 activation—therefore, cytostasis with early stage apoptosis!

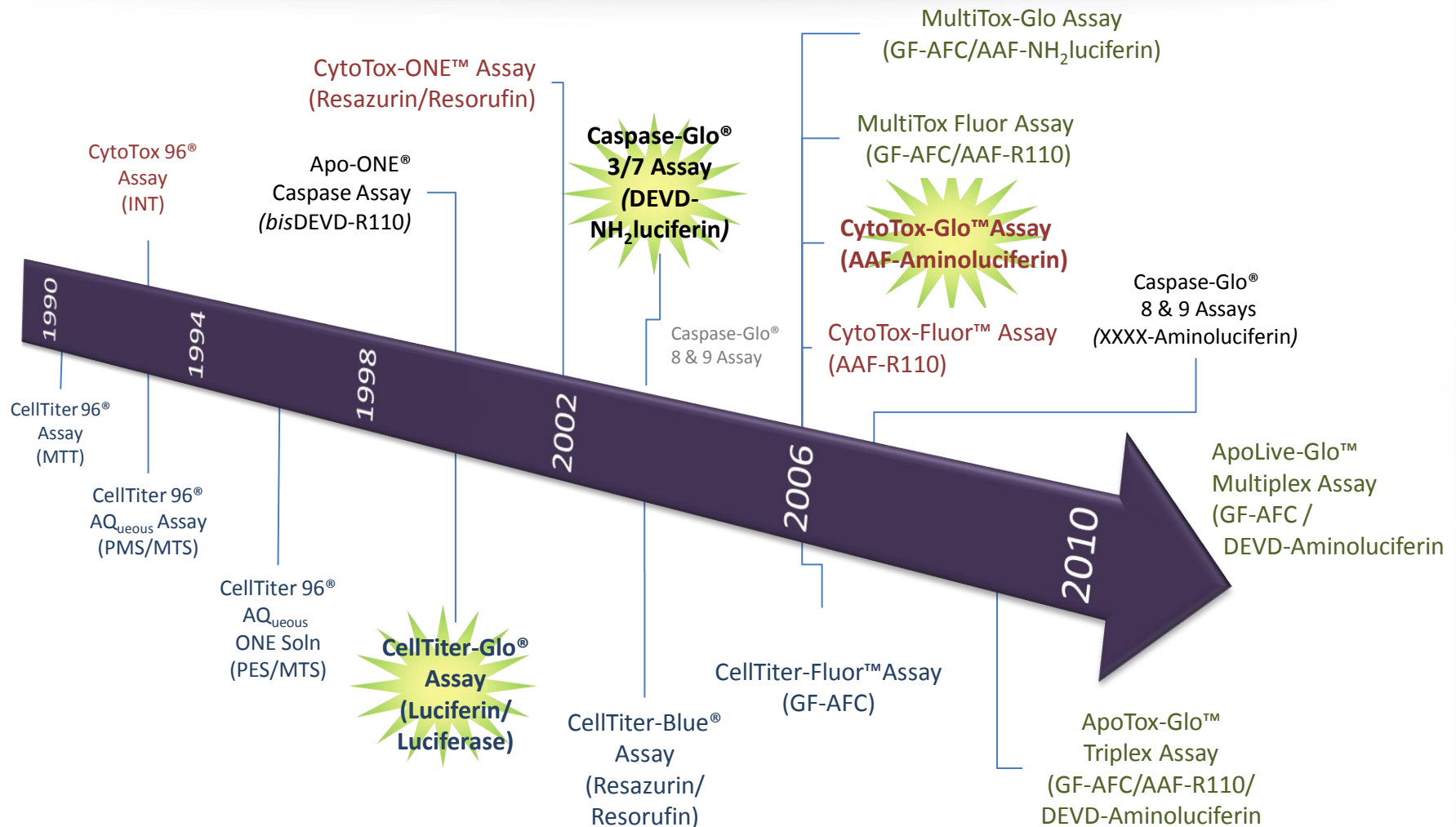
1. Reduce faulty interpretation or ambiguity from data sets
2. Eliminate variables from culturing duplicate or triplicate plates
3. Normalize data
4. Increase the content

***Plate-based assays for cell viability,
cytotoxicity, and caspase-dependent
apoptosis measurement***



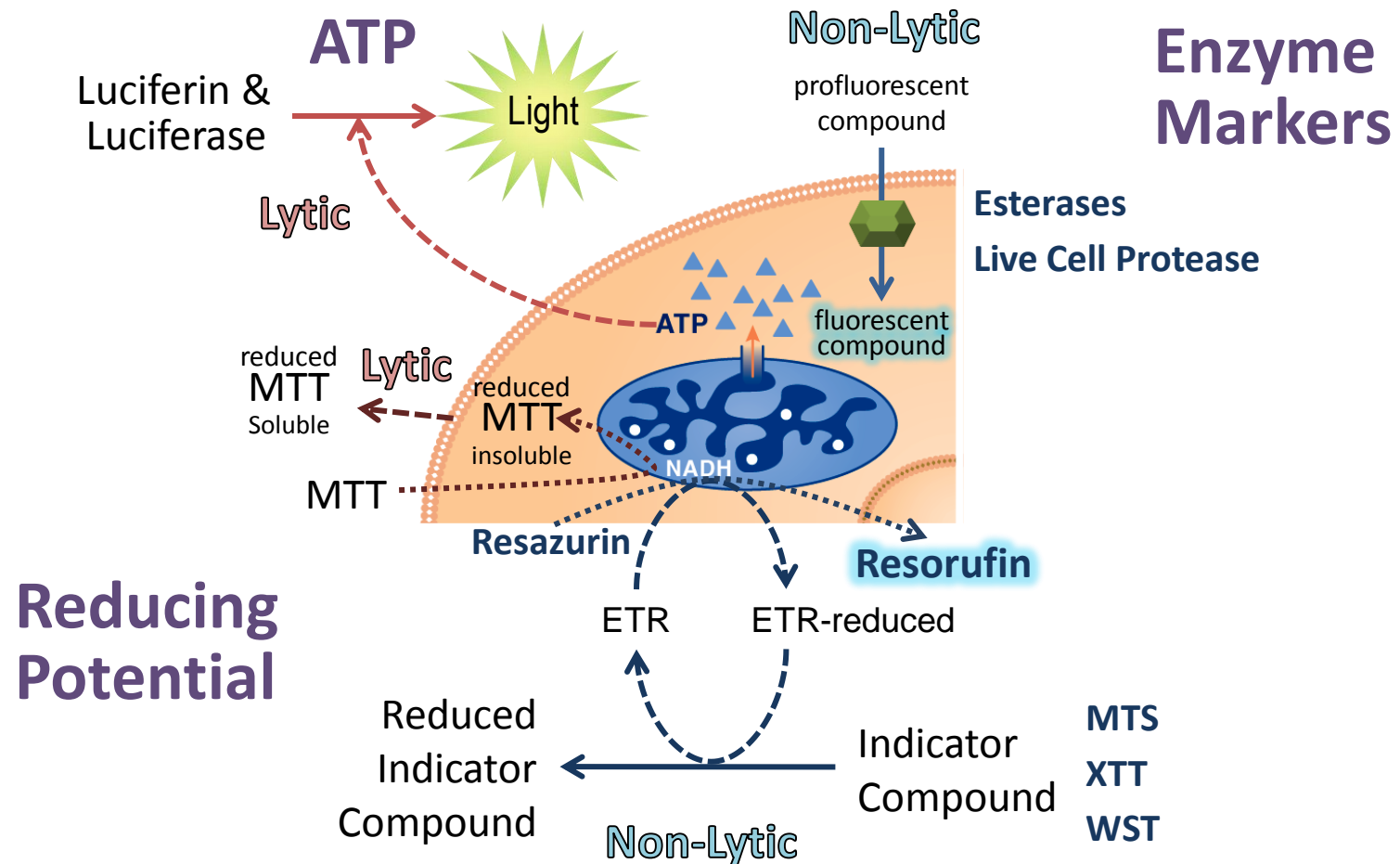


Development timeline for cell-based viability, cytotoxicity and apoptosis assays





Major methods to assess cell viability





Cell Viability Assays

Methods to measure live cells



Product	Steps	Time to results	Sensitivity	Measures	Equipment
<u>CellTiter-Glo® Luminescent Cell Viability Assay (Luciferase)</u>	3	10 minutes	++++ 10 cell sensitivity	ATP	Luminometer
<u>CellTiter-Fluor™ Cell Viability Assay (Gly-Phe-AFC)</u>	3	30 minutes	+++	Live Cell Protease	Fluorometer
Resazurin/Resorufin (fluorescent) <i>e.g., CellTiter-Blue™ Cell Viability Assay</i>	3	1-4 hours	++±	Reducing potential	Fluorometer
Soluble Formazan with Electron Transfer Reagent (MTS/XTT/WTS) <i>e.g., CellTiter 96® AQueous One Solution</i>	3	1-4 hours	++	Reducing potential	Spectrophotometer
Insoluble Formazan MTT <i>e.g., CellTiter 96® Assay</i>	5	1-4 hours	++	Reducing potential	Spectrophotometer
³ H-thymidine incorporation assay	8	2-24 hours	+++±	DNA Synthesis	Scintillation Counter

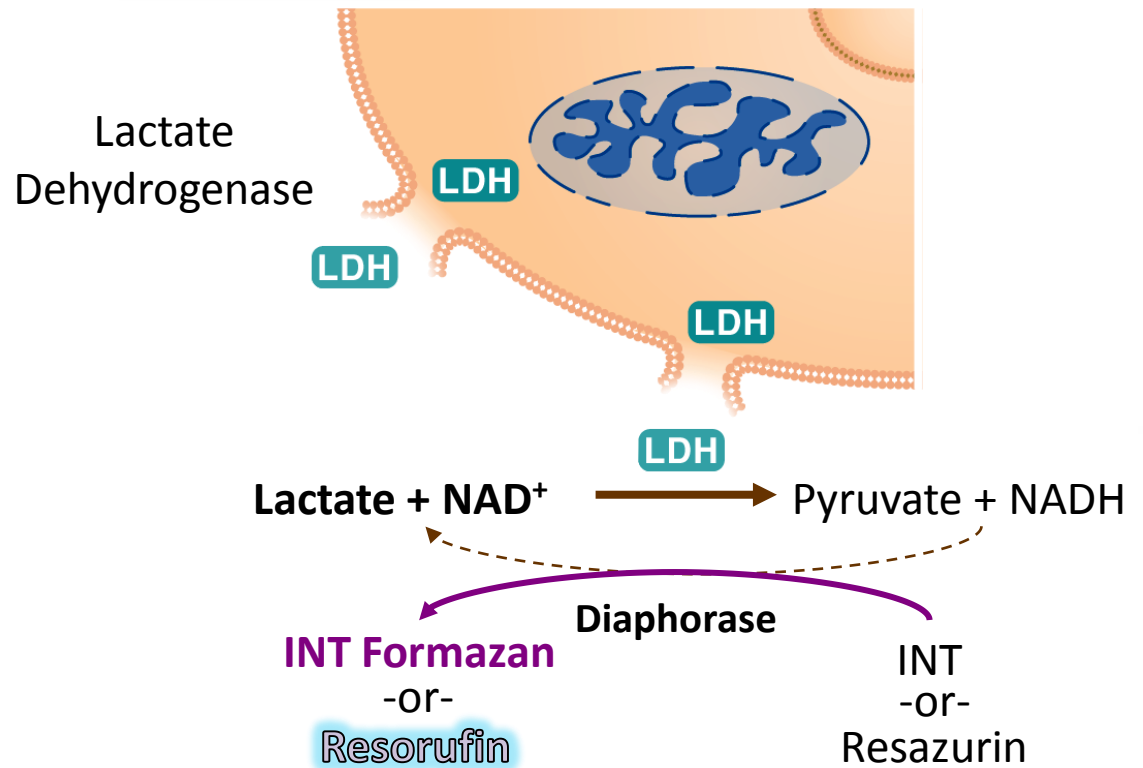


Cytotoxicity Assays

Assays are non-lytic.

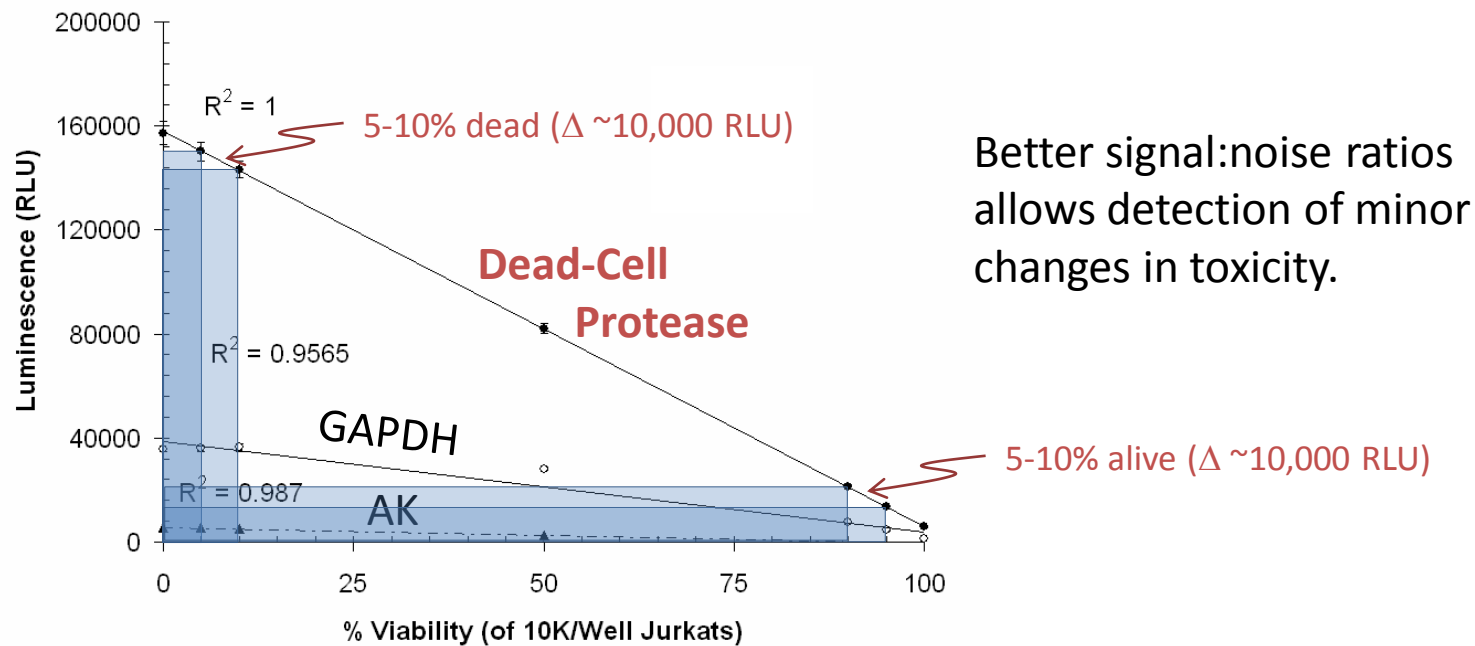
Other Enzymes for
luminescent output:

- Dead Cell Protease
- Adenylate Kinase
- Glyceraldehyde-3-PO₄-Dehydrogenase





Enough signal to differentiate 5-10% cytotoxicity



Mixture of live and sonicated cells
Plate read after 15 minutes of reagent addition




Major markers for cytotoxicity Assays

Marker	Method	Time	Marker Half-Life in Media	Direct Cell-Based Assay
Lactate Dehydrogenase (e.g., <u>CytoTox 96® Cytotoxicity Assay</u> & <u>CytoTox-ONE™ Membrane Integrity Assay</u>)	Colorimetric (Formazan Chemistry)	30 min.	~10hr	No
	Fluorescent (Resazurin/Resorufin)	10 min.		Yes
Dead Cell Protease (e.g., <u>CytoTox-Fluor™</u> & <u>CytoTox-Glo™</u> Cytotoxicity Assays)	Fluorescent	30 min.	~8 hr	Yes
	Luminescent	15 min.		Yes
Glyceraldehyde-3-PO₄-Dehydrogenase	Luminescent	5 min.	~4 hr	Yes
Adenylate Kinase	Luminescent	5 min.	~3 hr	No

Sensitivity

Luminescent > Fluorescent > Colorimetric

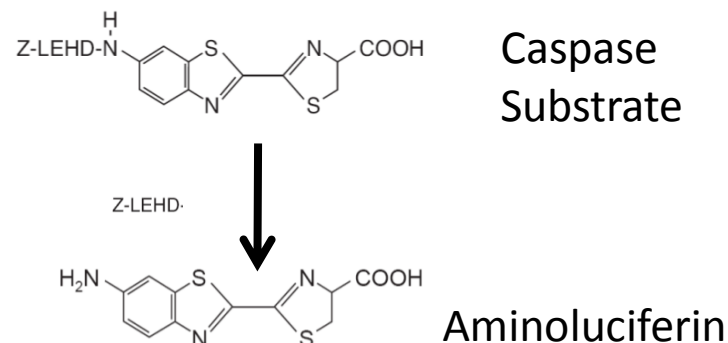
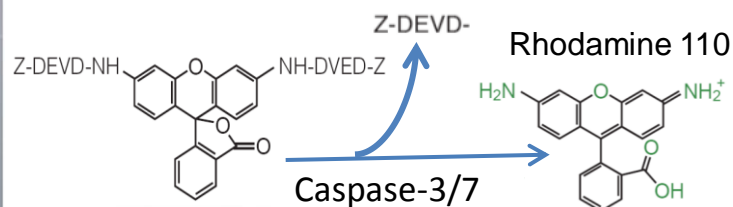
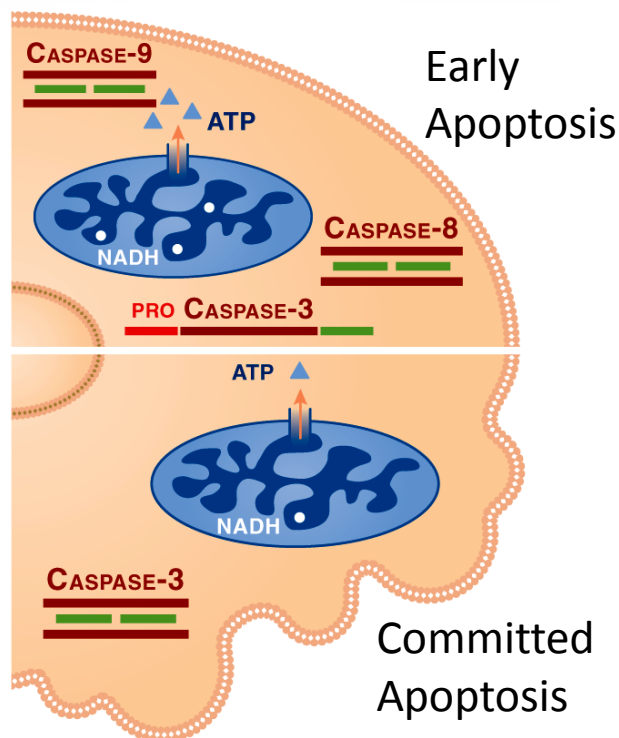


Governs how soon you have to assay after the cytotoxic event

Plate-based assays for caspase-dependent apoptosis



- Assays based on measuring activation of specific caspases.
- Cells with activated caspase-3/7 are considered committed to apoptotic cell death.
- Assays are lytic.



Caspase-Glo® 3/7 Assay

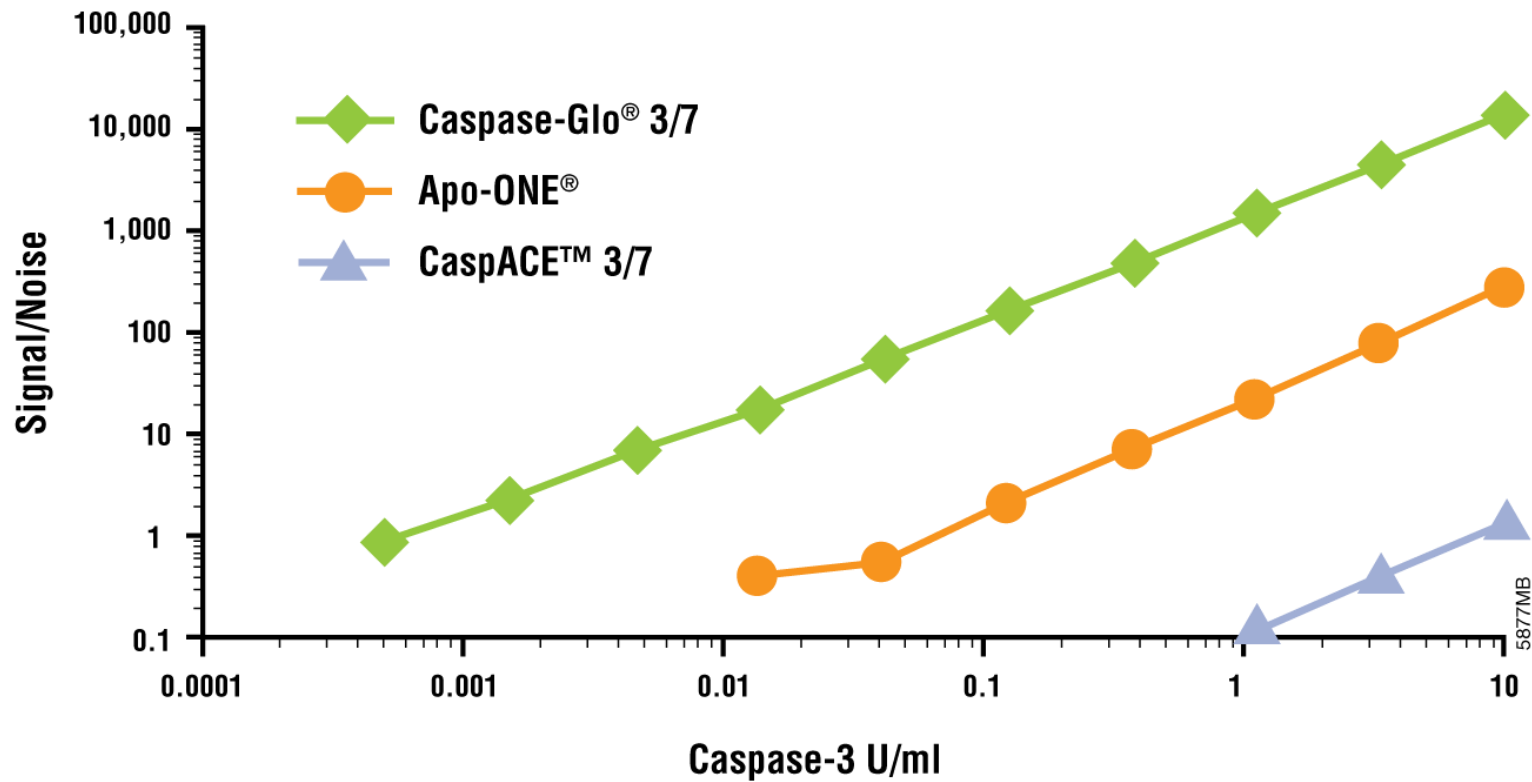
Caspase-Glo® 8 Assay

Caspase-Glo® 9 Assay

Apo-ONE® Caspase-3/7 Assay



Luminescence is most sensitive



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