

Multiplexing Cell-Based Assays

Get more biologically *relevant data*
from the same sample



Save Precious Time With Same-Well Multiplexing

Six Ways Multiplexing Can Enhance Your Research

- 1. Reduces variability:** Eliminate inconsistencies from replicate plates.
- 2. Enhances data accuracy:** Normalize results for clearer interpretation.
- 3. Provides a more complete picture:** Gain deeper insights into cellular responses.
- 4. Differentiates responses:** Distinguishes specific from general effects on cell health.
- 5. Saves time & costs:** Minimize cell culture time and reagent consumption.
- 6. Saves precious material:** Optimize the use of rare samples & test compounds.



1. Plate cells

Seed cells into a multiwell plate and incubate under appropriate conditions.

2. Add Assay #1 Reagent

e.g., CellTox™ Green Cytotoxicity Assay

3. Measure Assay #1

Read luminescent signal in a microplate luminometer.

4. Add Assay #2 Reagent

e.g., Caspase-Glo® 3/7 Assay

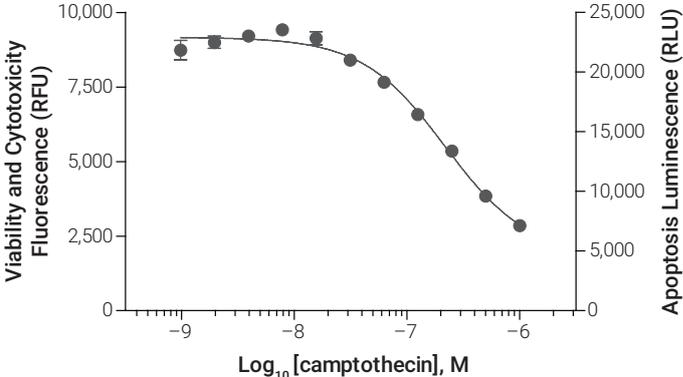
5. Measure Assay #2

Read luminescent signal in a microplate luminometer to generate an additional layer of biological insight from the same sample.

Things to Consider

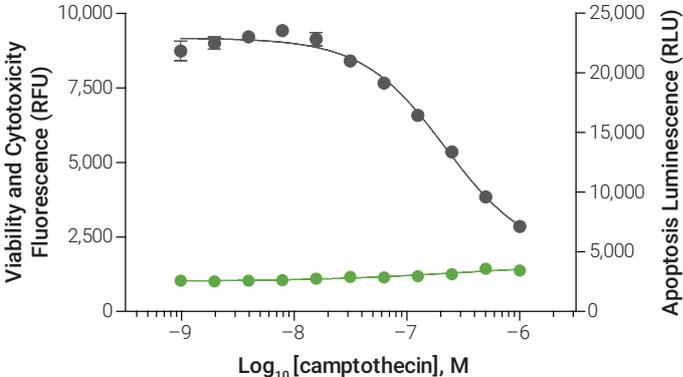
When considering multiplexing two or more assays, the assays must meet the following criteria:

1. The signals for the multiple assays must be spectrally or temporally distinct.
2. The assay chemistries must be compatible.
3. The assay volumes must fit into the same well or be easily separated (e.g., one assay uses the cells, the other uses the culture medium).



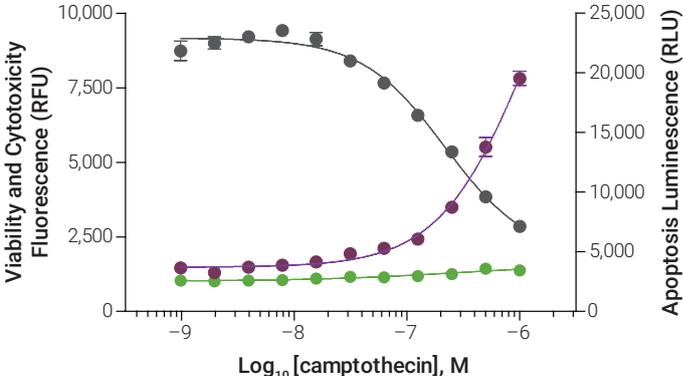
Assay #1: Viability

Conclusion 1:
The treatment appears to have a cytotoxic effect.



Assay #2: Cytotoxicity

Conclusion 2:
The treatment does not appear to be cytotoxic but rather cytostatic.



Assay #3: Apoptosis

Conclusion 3:
The treatment shows a cytostatic effect with induction of apoptosis.

An example of cytostasis seen using the ApoTox-Glo™ Triplex Assay. Camptothecin treatment of 10,000 K562 human erythroleukemia cells for 48 hours resulted in a dose-dependent decrease in apparent viability with no cytotoxicity, but an increase in caspase-3/7 activity, a profile consistent with cell cycle arrest and early phase apoptosis.

Multiplex And Normalize Data With Regard to *Dead Cells or Total Cells*

NL = NanoLuc® Luciferase

FL = Firefly Luciferase

✔ = Assays can be combined for multiplexing



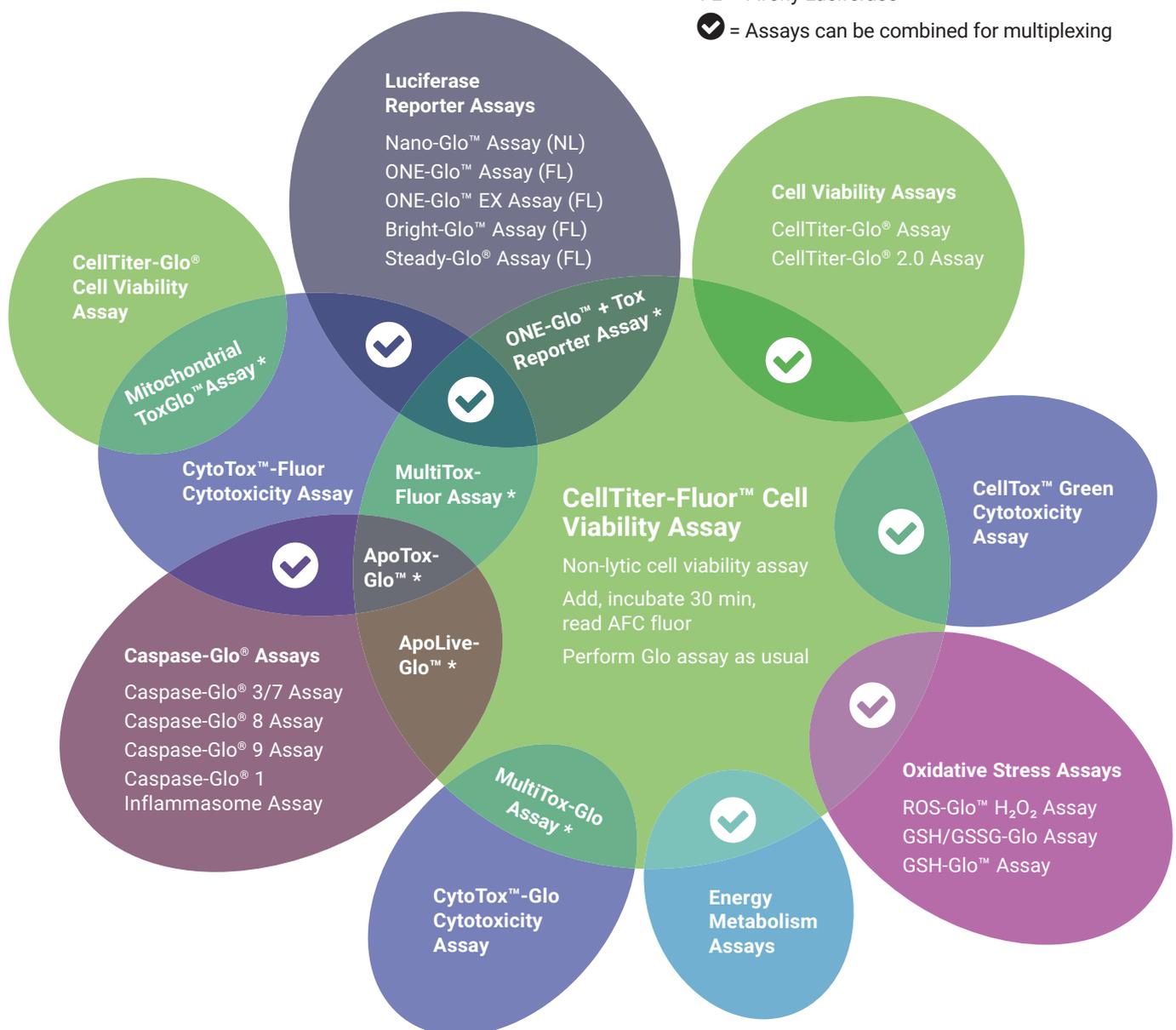
* Multiplexing kits contain several compatible assays, for details see table on page 5/6

Multiplex And Normalize Data With Regard to *Viable Cells*

NL = NanoLuc® Luciferase

FL = Firefly Luciferase

✔ = Assays can be combined for multiplexing



Learn more about cell-based assays for measuring cell viability, apoptosis and more:

www.promega.com/CellbasedAssays

* Multiplexing kits contain several compatible assays, for details see table on page 5/6

Multiplexing Guide

Compatible Assays at a Glance

1 Order matters – select this assay first

		First Assay												
		Viability					Cytotoxicity					Apoptosis		
		CellTiter-Glo®	CellTiter-Fluor™	CellTiter-Blue®	CellTiter 96® AQ	RealTime-Glo™ MT	CytoTox-Fluor™	CytoTox-Glo™	CellTox™ Green	CytoTox-ONE™	LDH-Glo®	Apo-ONE® Caspase 3/7	Caspase-Glo® 3/7	RealTime-Glo™ Annexin
Viability	CellTiter-Glo®		✓	✗	(✓)	✓	✓	(✓)	✓	✓	✓	✗	✗	✓
	CellTiter-Fluor™	✗		✗	✗	✗	✓	✓	✓	(✓)	✓	✗	✗	✓
	CellTiter-Blue®	✗	✗		✗	✗	(✓)	(✓)	✓	(✓)	(✓)	✗	✗	(✓)
	CellTiter 96® AQ	✗	✗	✗		✗	(✓)	(✓)	(✓)	✓	(✓)	✗	✗	(✓)
	RealTime-Glo™ MT	✗	✗	✗	✗		✓	(✓)	✓	(✓)	✓	✗	✗	✗
Cytotoxicity	CytoTox-Fluor™	✗	✓	✗	✗	✓		✗	✗	✗	(✓)	✗	✗	✗
	CytoTox-Glo™	✗	✓	✗	✗	(✓)	✗		(✓)	✗	(✓)	✗	✗	✗
	CellTox™ Green	✗	✓	✗	✗	✓	✗	(✓)		✗	✓	✗	✗	✓
	CytoTox-ONE™	✗	(✓)	✗	✗	(✓)	(✓)	(✓)	✓		✓	✗	✗	(✓)
	LDH-Glo®	✗	(✓)	(✓)	(✓)	(✓)	(✓)	✗	(✓)	✗		✗	✗	(✓)
Apoptosis	Apo-ONE® Caspase 3/7	✗	(✓)	✓	✗	(✓)	(✓)	(✓)	✗	✓	✓		✗	✗
	Caspase-Glo® 3/7	✗	✓	✗	(✓)	(✓)	✓	(✓)	✓	(✓)	✓	✗		✓
	RealTime-Glo™ Annexin	✗	(✓)	✗	(✓)	✗	(✓)	✗	✓	✗	(✓)	✗	✗	
Oxidative Stress	GSH/GSSG-Glo™	✗	✓	✗	(✓)	✗	✓	(✓)	✓	(✓)	✓	✗	✗	(✓)
	ROS-Glo™ H ₂ O ₂	✗	✓	✗	✗	✓	(✓)	(✓)	✓	(✓)	✓	✗	✗	(✓)
Metabolism	NAD(P)/NAD(P)H-Glo™	✗	✓	✗	(✓)	✓	✓	(✓)	(✓)	(✓)	✓	✗	✗	(✓)
	Glucose-Glo™	✗	✓	✗	(✓)	✓	✓	(✓)	(✓)	(✓)	✓	✗	✗	(✓)
	Lactate-Glo™	✗	✓	✗	(✓)	✓	✓	(✓)	(✓)	(✓)	✓	✗	✗	(✓)
	Metabolite-Glo™	✗	✓	✗	(✓)	✓	✓	(✓)	(✓)	(✓)	✓	✗	✗	(✓)
Reporter	Firefly (e.g., ONE-Glo™)	✗	✓	✗	(✓)	(✓)	✓	(✓)	✓	(✓)	✓	✗	✗	(✓)
	NanoLuc®	✗	✓	✗	(✓)	(✓)	✓	(✓)	✓	(✓)	✓	(✓)	✗	✗
	NanoBiT® (LgBiT:HiBiT)	✗	✓	✗	(✓)	(✓)	✓	(✓)	✓	(✓)	✓	(✓)	✗	✗
	Lumit® Immunoassays	✗	✓	✗	(✓)	(✓)	✓	(✓)	(✓)	(✓)	✓	✗	✗	✗

- ✓ Multiplexing is possible
- (✓) Multiplexing may be possible, with constraints; please contact our Technical Service
- ✗ Multiplexing is not possible

Please contact our Technical Service team under techserv@promega.com for expert guidance and information about additional multiplex options not listed.

Oxidative Stress		Metabolism				Reporter				Multiplex Kits					
GSH/GSSG-Glo™	ROS-Glo™ H ₂ O ₂	NAD(P)/NAD(P)H-Glo™	Glucose-Glo™	Lactate-Glo™	Metabolite-Glo™	Firefly (e.g., ONE-Glo™)	NanoLuc®	NanoBIT® (LgBiT:HiBiT)	Lumit® Immunoassays	ApoTox-Glo™ Triplex	ApoLive-Glo™	Mitochondrial Tox-Glo™	MultiTox-Fluor	MultiTox-Glo	ONE-Glo™ + Tox
x	✓	x	(✓)	(✓)	(✓)	x	(✓)	(✓)	(✓)						
x	(✓)	x	(✓)	(✓)	(✓)	x	(✓)	(✓)	(✓)						
x	(✓)	x	(✓)	(✓)	(✓)	x	(✓)	(✓)	(✓)						
x	(✓)	x	(✓)	(✓)	(✓)	x	(✓)	(✓)	(✓)						
x	✓	x	(✓)	(✓)	(✓)	x	x	(✓)	(✓)						
x	(✓)	x	(✓)	(✓)	(✓)	x	(✓)	(✓)	(✓)						
x	(✓)	x	(✓)	(✓)	(✓)	x	(✓)	(✓)	(✓)						
x	(✓)	x	(✓)	(✓)	(✓)	x	(✓)	(✓)	(✓)						
x	(✓)	x	(✓)	(✓)	(✓)	x	(✓)	(✓)	(✓)						
(✓)	(✓)	x	(✓)	(✓)	(✓)	x	(✓)	(✓)	(✓)						
x	(✓)	x	(✓)	(✓)	(✓)	x	(✓)	(✓)	(✓)						
x	(✓)	x	(✓)	(✓)	(✓)	x	x	x	(✓)						
	(✓)	x	(✓)	(✓)	(✓)	x	(✓)	(✓)	(✓)						
x		x	(✓)	(✓)	(✓)	x	(✓)	(✓)	(✓)						
x	(✓)		(✓)	(✓)	(✓)	x	(✓)	(✓)	(✓)						
x	(✓)	x		(✓)	(✓)	x	(✓)	(✓)	(✓)						
x	(✓)	x	(✓)		(✓)	x	(✓)	(✓)	(✓)						
x	(✓)	x	(✓)	(✓)	(✓)		x	x	(✓)						
x	(✓)	x	(✓)	(✓)	(✓)	✓		x	(✓)						
x	(✓)	x	(✓)	(✓)	(✓)	✓	x		(✓)						
x	(✓)	x	(✓)	(✓)	(✓)	x	x	x							

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