Determine predictive mechanisms of toxicity using a single-well multiplexed assay

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Multiplexing can be defined as the use of compatible assays to measure multiple parameters in a single well. One significant advantage, particularly in secondary screening, is generating more biologically relevant data. Information about cell health, toxicity and apoptosis events from the same population of cells provides a broader picture of what is occurring inside cells and is particularly well suited for in vitro toxicology applications.

The assay simplifies multiplexing

The ApoTox-Glo™ Triplex Assay is designed to measure cell viability, cytotoxicity, and caspase activation in each well in common multiwell plates. The assay measures two protease biomarker activities, a live-cell marker (cell viability) and a dead-cell marker (cytotoxicity), as illustrated in **Figure 1**. The live-cell protease activity is restricted to intact viable cells and is measured using a fluorogenic, cell-permeant substrate, which enters intact cells and is cleaved by the live-cell protease. A second, distinct fluorogenic cell-impermeant substrate is used to measure dead-cell protease activity. Dead-cell proteases are released from cells that have lost membrane integrity. The live- and dead-cell proteases produce different fluorescent products with distinct excitation and emission spectra allowing them to be detected simultaneously[1] using a fluorescent plate reader.

Caspase activity is measured using a luminogenic substrate in a subsequent step in the assay. The luminescent signal is proportional to the amount of caspase activity present. Assay configurations for multiwell plate formats are provided in **Table 1**.

Performance of the assay

Cell viability, cytotoxicity, and caspase-3/7 activity were assessed using the assay in K562, Jurkat, L929, and HepG2 cell lines treated with mechanistically different cytotoxic compounds (ionomycin, staurosporine, bortezomib and SAHA). The cytotoxic profiles generated using the assay demonstrate the expected trends for the effect of each compound on the cells (**Figure 2** and http://www.promega.com/pubs/tpub_011.htm). *In vitro* cytotoxicity is dependent upon dosage of compound and time of exposure. The kinetics of measurable cytotoxic biomarkers can vary widely between individual compounds and treatments. Therefore, it is important to characterise new compounds using multiple exposure periods.

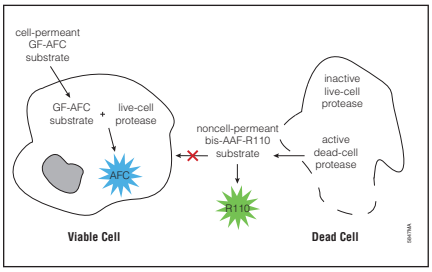

Figure 1: Viability and cytotoxicity are measured as fluorescent readouts in the first step of the ApoTox-Glo™ Assay. A second step measures caspase activity as a bioluminescent readout

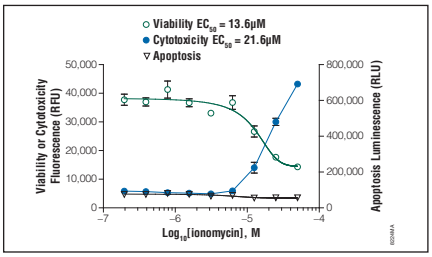

Figure 2: Representative data of the ApoTox-Glo™ Triplex Assay. K562 cells in suspension were treated for four hours with ionomycin in 96-well format resulting in a dose-dependent decrease in viability and increase in cytotoxicity with no caspase-3/7 activation, which is consistent with primary necrosis

Conclusion

Multiplexing with the Promega ApoTox-Glo™ Triplex Assay helps to provide researchers with information about cell health, toxicity and apoptosis events, while minimising the assay workflow time. The assay is easily configured to work across multiwell plate formats and serves as an especially useful tool to better understand and predict the mechanism of cellular cytotoxicity.

	96-WELL	384-WELL	1536-WELL
Recommended cell density (cells/well)	10,000-20,000	5,000-10,000	1,000-5,000
Add cells + cmpds in culture medium	100µl	20µl	4µl
	Treat cells at 37°C in 5% CO_2 for the desired amount of time (ie, 4-48 hrs)		
Add viability/ cytotoxicity reagent	20µl	5µl	1.25µl
	Incubate at 37°C for 30 minutes. Measure fluorescence		
Add Caspase-Glo® 3/7 Reagent	100µl	25µl	4µl
	Incubate at room temperature for 30 minutes. Measure luminescence		

Table 1: ApoTox-Glo™ Triplex Assay protocol incorporates a simple sequential 'add-mix-read' format. The volumes of each assay component can be scaled to meet varying researchers' throughput needs and is highly amenable to automation

Reference

1 Niles, AL et al (2007). A homogeneous assay to measure live and dead cells in the same sample by detecting different protease markers. Anal. Biochem 366, 197–206.