



# MultiTox-Fluor Multiplex Cytotoxicity Assay for the Breast Cancer Cell line, MCF7, Using the BioRAPTR FRD™ Microfluidic Workstation and PARADIGM™ Detection Platform

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## ABSTRACT

The challenge of assessing the safety of the expanding pool of novel compounds under consideration as possible therapeutic agents, while concomitantly reducing the costs of drug discovery demands the ability to rapidly measure cellular viability and cytotoxicity. The protocols described in this poster were used to automate the MultiTox-Fluor Multiplex Cytotoxicity Assay from Promega Corporation to measure the number of both live and dead cells in the breast cancer cell line, MCF7, after exposure to cytotoxic compounds. The MultiTox-Fluor assay utilizes two fluorogenic peptide substrates which recognize cell viability and cytotoxicity markers. The activities of two proteases are simultaneously detected, producing ratiometric and inversely-correlated measures of cell viability and cytotoxicity.

The BioRAPTR FRD workstation from Beckman Coulter was used to automate the cell, compound, and reagent dispensing for the MultiTox-Fluor assay in a small volume 384-well microplate format. The detection and analysis were performed on the PARADIGM Detection Platform from Beckman Coulter, using a new cartridge specifically designed for MultiTox-Fluor Multiplex Cytotoxicity Assay applications.

Note: The BioRAPTR FRD and PARADIGM Detection Platform are for Research Use Only; not for use in diagnostic procedures.

## INTRODUCTION

### BioRAPTR FRD Microfluidic Workstation



The BioRAPTR FRD (Flying Reagent Dispenser) Microfluidic Workstation provides non-contact liquid handling and is capable of rapidly and accurately dispensing various liquids including cells with volume ranges from 0.1 to 60 µL in 384-, 1536- or 3456-well plates. It is able to dispense the cells with a high degree of precision while maintaining cell viability during the entire process.

### PARADIGM Detection Platform

The PARADIGM Detection Platform utilizes a patent-pending design that allows for real-time system configuration by the user in less than five minutes.

#### PARADIGM Detection Platform features:

- User-friendly integration and user-configurable modular design
- Detection of Multiple formats plates ranging from 6- to 1536 wells
- Multiple detection modes: Absorbance, HTRF, TRF, FP, FI, FRET and Glow LUM.
- On-the-fly detection with intelligent cartridge identification



#### Cartridges for Broad Array of Detections:

- Absorbance
- Multimode
- Fluorescence Intensity (fluorescein - rhodamine)
- Fluorescence Polarization (fluorescein)
- Cisbio HTRF™
- Invitrogen GeneBLAzer™
- Invitrogen™ LanthaScreen™
- PerkinElmer AlphaScreen™
- Promega MultiTox-Fluor Multiplex Cytotoxicity

The detection cartridge utilized in this poster is the MultiTox-Fluor Cartridge which is specifically designed for MultiTox-Fluor Multiplex Cytotoxicity Assay applications.

## ASSAY PRINCIPLE

The MultiTox-Fluor Cytotoxicity Assay simultaneously measures the relative number of live and dead cells based on their protease activities. As shown in Figure 3, the assay provides two fluorescent substrates which have different excitation and emission spectra.

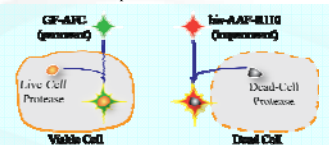


Figure 1. Principle of MultiTox-Fluor Cytotoxicity Assay.

The cell-permeant GF-AFC substrate enters intact cells and is cleaved by the viable cell protease to generate a fluorescence signal proportional to the number of living cells; the cell-impermeant substrate bis-AAF-R110 binds the dead-cell protease, released from cells that have lost membrane integrity and generates fluorescence signal proportional to the number of dead cells.

## BIORAPTR FRD WORKSTATION SETUP

### BioRAPTR 8-Tip Head Setup

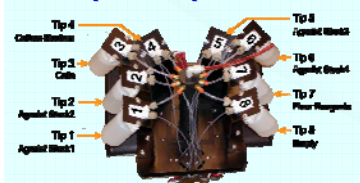


Figure 2. The MultiTox-Fluor Cytotoxicity Assay setup for the 8-tip BioRAPTR FRD workstation. Tip 1-4 were used for agonists dispensing, including digitonin, ionomycin and vinblastine (VBL).

### BioRAPTR Dispensing Tables

A. Digitonin Stock1: 2 mg/mL, dispense to make final 0.3, 0.1 and 0.03 mg/mL. Digitonin Stock2: 20 mg/mL, dispense to make final 10, 3, 1, 0.3 and 0 mg/mL.

B. Ionomycin Stock1: 60 nM, dispense to make final 30, 15, 10 and 5 nM. Ionomycin Stock2: 6 nM, dispense to make final 3, 1.5, 0.3 and 0 nM.

VBL Stock1: 2 mM, dispense to make final 800 nM. VBL Stock2: 1 mM, dispense to make final 400, 200, 100, 40, 20 and 10 nM.

Figure 3. The MultiTox-Fluor Cytotoxicity assay dispensing tables for cells, agonist stocks: digitonin (Figure 3A, Tip 1&2), ionomycin (Figure 3B, Tip 1&2) and vinblastine (Figure 3B, Tip 5&6), and assay kit reagents to make the final reaction volume of 20 µL per well.

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## RESULTS

### Digitonin Induced Cytotoxicity in Densities of MCF7 Cells

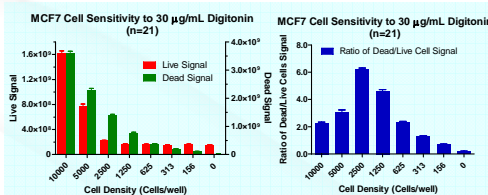


Figure 4. Effects of 30 µg/mL Digitonin on different MCF7 cells densities. Viable cell signals were detected at excitation 406 nm and emission 465 nm; dead cell signals were detected at excitation 504 nm and emission 542 nm. The ratios of dead cell to live cell signals were plotted on the right. Each bar represents 21 replicates.

MCF7 cells were dispensed into a white small volume 384-well microplate at different densities ranging from 0 to 10,000 cells/well by the 8-tip BioRAPTR FRD workstation, as illustrated in Figure 3A, Tip 3. Digitonin stock solution 2 mg/mL was dispensed into each well at a dispensing volume of 0.15 µL per well to make the final working concentration of 30 µg/mL digitonin and the plate was incubated for 10 min before reading on the PARADIGM platform using the MultiTox-Fluor detection cartridge. As shown in Figure 4, cell densities from 2,500 to 10,000 cells/well generated the best signal for live cells, dead cells and the ratio of live to dead signals. The density of 5,000 cell/well was chosen for the following cytotoxicity assay.

### Digitonin Induced Cytotoxicity in MCF7 Cells

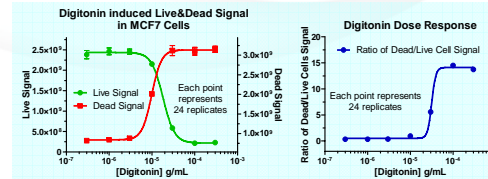


Figure 5. Digitonin (glycoside, which permeabilizes cell membrane) dose responses in MCF7 cells. Left: Superpose of cell viability and cytotoxicity response curves; Right: Ratio of dead/live cell fluorescence. Each point represents 48 replicates.

Assay Type	Z'	LogEC50	CV%	Replicates
Viability	0.72	-4.67 ± 0.02	2.2%	n=24
Cytotoxicity	0.64	-5.03 ± 0.01	1.4%	n=24
Ratio of Dead/Live	0.86	-4.32 ± 0.02	2.4%	n=24

Z' factor is the statistical parameter used to evaluate how much the dynamic range of an assay exists outside the 99% confidence intervals for the positive and negative controls. It indicates how small an effect in an assay can be reliably ascertained relative to data variation. Z-factor can be computed from the mean ( $\mu$ ) and standard deviation ( $\sigma$ ) of both the positive ( $\mu_p, \sigma_p$ ) and negative ( $\mu_n, \sigma_n$ ) controls, respectively. Z' ranges from 0 to 1.0, which between 0 and 0.5 is marginal; between 0.5 and 1.0 is an excellent assay.

MCF7 cells were dispensed into a small volume 384-well plate at the density of 5,000 cells/well in a volume of 5 µL by the 8-tip BioRAPTR FRD workstation. Digitonin were then dispensed at concentrations ranging from 0 to 0.3 mg/mL by two stock solutions, 2 mg/mL and 20 µg/mL, as illustrated in Figure 5. After 10 min incubation, the decrease of live signal and increase of dead signal were detected on the PARADIGM platform using the MultiTox-Fluor detection cartridge at 365<sub>Ex</sub>/405<sub>Em</sub> and 485<sub>Ex</sub>/505<sub>Em</sub> nm, simultaneously.

### Ionomycin Induced Cytotoxicity in MCF7 Cells

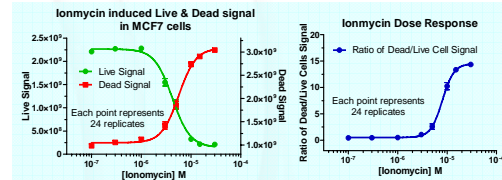


Figure 6. Ionomycin dose responses in MCF7 cells. Left: Superpose of live cell and dead cell signals; Right: Ratio of dead/live signals. Each point represents 24 replicates.

Assay Type	Z'	LogEC50	CV%	Replicates
Viability	0.79	-5.39 ± 0.03	2.3%	n=24
Cytotoxicity	0.64	-5.26 ± 0.02	2.3%	n=24
Ratio of Dead/Live	0.86	-5.10 ± 0.02	2.3%	n=24

### Vinblastine (VBL) Induced Cytotoxicity in MCF7 Cells

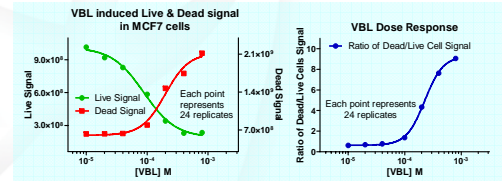


Figure 7. Vinblastine dose responses in MCF7 cells. Left: Superpose of live cell and dead cell signals; Right: Ratio of dead/live signals. Each point represents 24 replicates.

Assay Type	Z'	LogEC50	CV%	Replicates
Viability	0.61	-4.10 ± 0.03	4.4%	n=24
Cytotoxicity	0.69	-3.67 ± 0.02	2.3%	n=24
Ratio of Dead/Live	0.78	-3.61 ± 0.01	0.9%	n=24

MCF7 cells were dispensed into a small volume 384-well plate at the density of 5,000 cells/well in a volume of 5 µL. Ionomycin and VBL were then dispensed at concentrations ranging from 0.1 to 30 µM (ionomycin, Figure 3B & 6) and 10 to 800 nM (VBL, Figure 3B & 7). After 6 hrs incubation, the decrease of live cell signal and increase of dead cell signal were detected on the PARADIGM platform using MultiTox-Fluor detection cartridge. The data suggest that ionomycin, a calcium ionophore, can induce MCF7 cell apoptosis at the EC50 of 7.9 µM; VBL, an anti-cancer alkaloid which causes mitotic arrest by inhibiting tubulin polymerization, can induce MCF7 cell apoptosis at the EC50 of 0.25 nM after 6 hrs incubation.

## CONCLUSIONS

- The BioRAPTR FRD workstation and MultiTox-Fluor Cytotoxicity Detection Cartridge were successfully used for automation and detection of the cell-based MultiTox-Fluor Cytotoxicity assays in small volume 384-well plate format.
- The data show that the combination of BioRAPTR FRD workstation and PARADIGM MultiTox-Fluor Detection Cartridge is capable of rapidly and precisely performing the MultiTox-Fluor Cytotoxicity assay with low %CV value and Z' values greater than 0.5.

### Labware and Consumables

Materials	Manufacturer	Part No.
<b>Hardware</b>		
BioRAPTR FRD Workstation 8-Tip Head	Beckman Coulter	B18492
PARADIGM HTRF Configuration	Beckman Coulter	A41573
<b>Labware</b>		
Laboratory Bottles, High-Density PE, Wide Mouth, 30 mL	Nalgene	2104-0001
Laboratory Bottles, High-Density PE, Wide Mouth, 60 mL	Nalgene	2104-0002
CellStar, 384W SV Plate, PS, TC, ST, LoBase, WHT	Greiner Bio-One	788073
<b>Reagents</b>		
MultiTox-Fluor Multiplex Cytotoxicity Assay Kit	Promega Inc.	G92002