Combining CyBi®-FeliX Automated Liquid Handling, Cell Health Assays and Frozen, Thaw-and-Use Cells Enables Automated Cytotoxicity Profiling

Matt Butzler[1], Tracy Worzella[1], Marlon Hungriano[2], Felix Osorio[2], Ian Hanegraaff[2] and Cristopher Cowan[1]

[1]Promega Corporation, 2800 Woods Hollow Rd, Madison, WI 53711 and [2]Analytik Jena US, CyBio Product Line - Woburn, MA

1. Introduction

One biomarker alone cannot be used to determine the mechanism of cytotoxicity. Combining biomarkers associated with membrane integrity changes (cytotoxicity), cell number and apoptosis can more closely distinguish populations of cells that are necrotic, apoptotic, or growth arrested.

Automation can help facilitate the process of compound profiling by providing a fast, precise, and consistent manner to carry out experiments. The automated process that we describe enables mechanistic toxicity profiling of up to eight compounds in quadruplicate in 384-well microplate format.

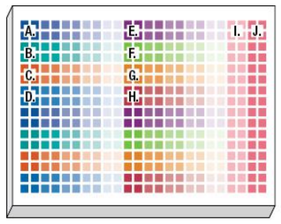


Figure 1. Plate layout for 384-well profiling.

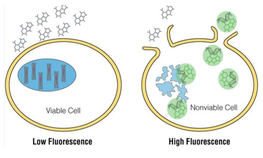
2. Features of the CyBi®-FeliX Automated Liquid Handler

Notable features of the configuration tested:

- **Size:** 12 deck positions on two levels in a small footprint of 650mm x 450mm (W x D).
- **Versatility:** Interchange a 96-channel pipetting head (250µl) with a 8-channel liquid handling adapter.
- **Multifunctional:** Automated swapping to the 8-channel adapter for smooth transition from cell dispense to serial titration.
- **Speed:** Quick dispensing by 96-channel head to 384-well assay plate negates worries of cell settling in the source reservoir or long term exposure of cells to the environment.

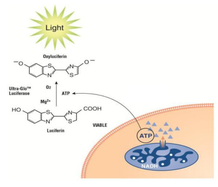
Figure 2. The CyBi®-FeliX possesses a small footprint with sufficient deck positions for reagents and labware consumables. Users may customize the instrument configuration to meet their specific needs.

3. Detection Reagents Measure Cytotoxicity, Cell Number and Apoptosis



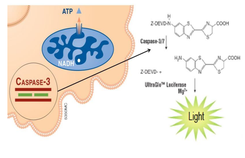
CellTox™ Green:
A non-lytic dye reagent that binds to DNA of nonviable cells.

Fluorescence is proportional to dead cell number.



CellTiter-Glo® 2.0:
A lytic reagent that quantifies ATP content in a firefly luciferase reaction.

Luminescence is proportional to viable cell number.



Caspase-Glo® 3/7:
A lytic reagent comprised of a Z-DEVD pro-luciferin substrate that is cleaved by caspase-3/7 to produce luciferin (quantified in a firefly luciferase reaction).

Luminescence is proportional to caspase-3/7 activity.

Figure 3. The profiling panel of assays.

4. Using Multiple CyBi®-FeliX Pipetting Adapters Allows for Rapid Automation of Experimental Steps

Liquid Handling Step	Head or Adapter used	Approximate time to complete task
1. Multi-dispense cells to 384-well assay plate	96-channel head	10 seconds
2. Transfer diluent to compound titration plate.	8-channel adapter	1 minute
3. Titrate test compounds	8-channel adapter	2 minutes
4. Multi-dispense titrated compounds to assay plate	96-channel head	10 seconds
5. Multi-dispense detection reagents to assay plate	96-channel head	10 seconds

5. Excellent Z´-Factor Scores are Achieved with All Assays

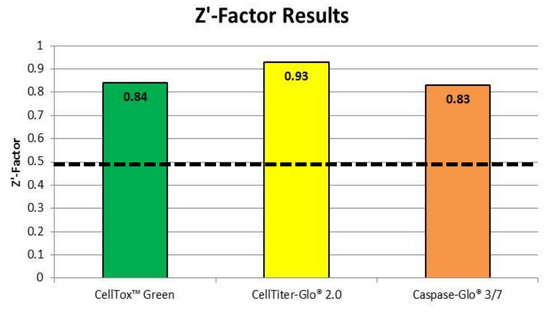


Figure 4. Z'-Factor scores > 0.5 represent excellent assay quality and precise liquid handling. Cells were treated with cytotoxins or DMSO control for 24 hours. Fluorescent and luminescent signals were quantified with the GloMax® Discover plate reader. Z'-Factor (*Zhang,et.al.*1999).

6. Frozen, Thaw-and-use Cells are a Seamless Fit in the Mechanistic Toxicity Profiling Workflow

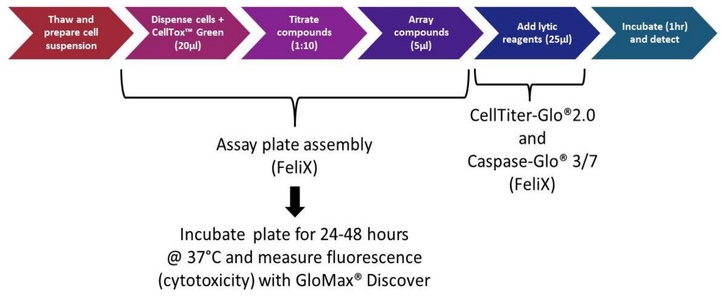


Figure 5. The toxicity profiling workflow. Using Frozen Instant HepG2 cells from CCS (Cell Culture Service) eliminates the need for cell culture.

7. Representative Mechanistic Toxicity Profiles for Apoptosis, Necrosis, and Growth Arrest

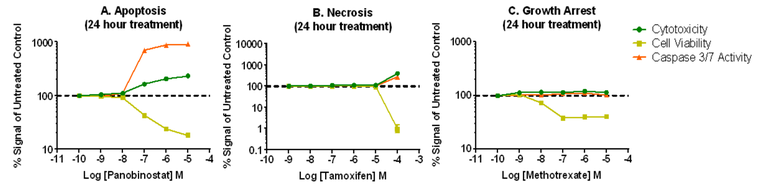


Figure 6. Mechanistic cytotoxicity profiles demonstrate (A) apoptosis, (B) necrosis, and (C) growth arrest of HepG2 cells at 24 treatment. The apoptosis profile (A) shows a hallmark dose-dependent increase in caspase-3/7 activity with corresponding decrease in the cell viability marker, ATP. A small increase in the cytotoxicity biomarker indicates the onset of secondary necrosis. The necrosis profile (B) is consistent with membrane integrity changes and profound decrease in cell viability at high concentration. Membrane integrity changes along with low level caspase detection suggest that apoptosis did occur much earlier and that 24 hour time point is outside the window of maximum detection. The growth arrest profile (C) shows a potent dose-dependent effect on the cell viability biomarker with no change in caspase and membrane integrity biomarkers, indicating an anti-proliferative effect.

8. Compound Exposure Time is Important When Assessing Mechanistic Cytotoxicity

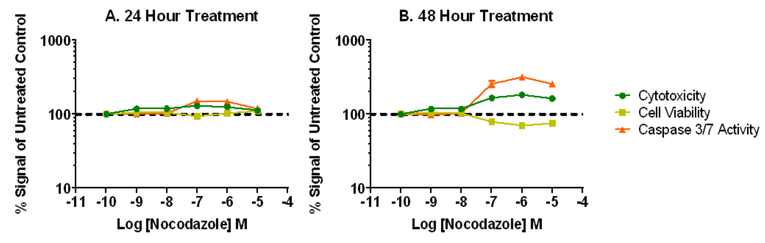


Figure 7. Time and dose-dependent effects of Nocodazole on HepG2 cell health. No biomarker changes are detected 24 hours post-treatment (A), suggesting that cells remain viable in culture. At 48 hours (B) a biologically different profile is observed. Dose-dependent decreases in cell viability with concordant increases in caspase-3/7 activity and cytotoxicity indicate cells progressing through apoptosis and secondary necrosis.

9. Conclusions

Automation provides a fast and robust method of setup for 384-well cell profiling experiments:

- 96- and 8-channel pipetting capabilities of the CyBi®-FeliX allow for automation of all steps of experimental setup and reagent addition.
- Excellent Z'-scores reflect precise liquid handling and assay quality.

Multiplexing conserves cells and consumables:

- Three biomarker readouts are achieved on every plate of cells.
- More data are acquired per experiment.

Expected biological profiles confirm the integrity of the method presented here:

- Panobinostat (apoptosis), Tamoxifen (necrosis), and Methotrexate (growth arrest) affected HepG2 cell health as expected.
- Time course studies with Nocodazole demonstrate the importance of both dose and exposure time.

Corresponding author: tracy.worzella@promega.com