Profiling the Mechanism of Cytotoxicity Using the Gilson PIPETMAX® 268

APPLICATION NOTE 1033

KEYWORDS

Cytoxicity, Cell viability, mechanistic toxicity profiling, PIPETMAX® 268

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INTRODUCTION

The drug target and other impacts of a therapeutic agent on the model system (Figure 1) typically dictate the design of the cytotoxicity studies. Regardless of drug-cell model, there are numerous cell culture and assay variables that need to be considered in establishing a cytotoxicity assay that meets the translational research requirements of 'verifiable science' and generates data that are reproducible. The cell culture requirements include the:

- Number of cells per well and the equilibration period prior to the assay may affect cellular physiology
- Maintenance and handling of stock cultures at each step of the manufacturing process should be standardized and validated for consistency
- Assay responsiveness to test compounds can be influenced by many subtle factors including culture medium surface-to-volume ratio, gas exchange, evaporation of liquids and edge effects.

In addition, there are important assay-specific factors that need to be considered. These include:

- Pipetting reproducibility and consistency
- Sample preparation accuracy within and between the numerous samples being analyzed

The PIPETMAX 268, the automated pipetting platform for maximizing reproducibility of biological sample preparation, was used to demonstrate the reproducibility and accuracy for a cell-based mechanistic toxicity profiling application. Dispensing the cells, compounds and reagents necessary to conduct the following assays in 384-well format, the PIPETMAX was used to perform the following assays: Promega CellTiter-Glo® Luminescent Cell Viability Assay, Promega MultiTox-Fluor Multiplex Cytotoxicity Assay, Promega Caspase- Glo® 3/7 Assay and CellTox™ Green Cytotoxicity Assay.

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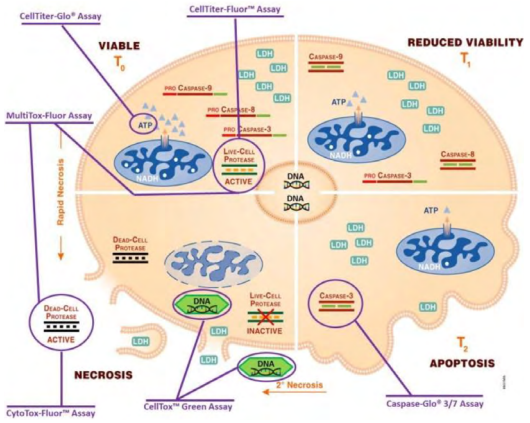


Figure 1
Numerous cytotoxicity impacts on a typical model system

MATERIALS AND METHODS

PIPETMAX 268, two pipetting heads – 8x20 and an 8x200

K562 cells

Bortezomib, a proteasome inhibitor

Ionomycin, a Ca2+ ionophore

Promega CellTiter-Glo® Luminescent Cell Viability Assay

Promega MultiTox-Fluor Multiplex Cytotoxicity Assay

Promega Caspase- Glo® 3/7 Assay

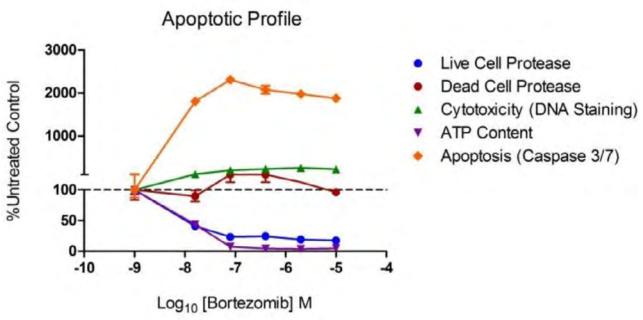
CellTox™ Green Cytotoxicity Assay

The PIPETMAX 268 was set up with a standard tray and two pipetting heads, an 8x20 and an 8x200. The bed was configured with two D200 tip racks, a 12-column or 8-row reservoir, a 96-well dilution plate and two 384-well assay plates. The cell-based assay workflow outlined in Figure 2 was used for the set-up of these assays.

Figure 2
Cell-based assay workflow

RESULTS

A.



B.

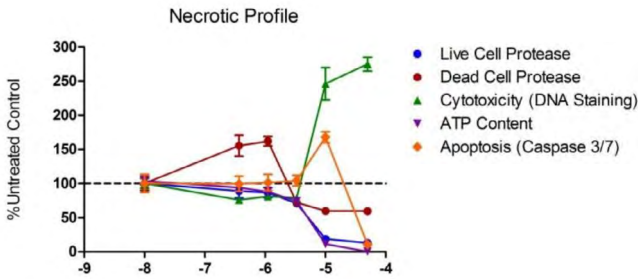


Figure 3
Results from 24 Hour Treatment of K562 Cells:

A. Apoptotic profile: Bortezomib, a proteasome inhibitor

B. Necrotic profile: Ionomycin, a Ca2+ ionophore

Within 24 hours of treatment of K562 cells with the proteasome inhibitor, Bortezomib, apoptosis had occurred as seen by the large dose-dependent induction of caspase-3/7 (Figure 3A) with corresponding decrease in ATP and live cell protease cell viability markers. Membrane breakdown was initiated as measured by small increases in DNA staining and dead cell protease activity.

The necrotic profile, post treatment with Ionomycin (Figure 3B) showed dose-dependent changes in membrane integrity as measured by an increase in DNA staining and detection of dead cell protease activity at lower concentrations.

Concordant decreases in cell viability markers were noted. Decreases in caspase-3/7 and dead cell protease activity at high concentrations were a result of biomarker decay at the time of measurement, brought on by rapid induction of necrosis.

SUMMARY

PIPETMAX is capable of performing microplate-based cell viability and toxicity assays. The instrument has capabilities for:

- Standard assay setup including compound titration, cell and reagent addition
- Unattended protocol operation to free up researcher time for other responsibilities
- Multi-well plate dispensing for 96-well and 384-well formats
- Broad volume range capabilities to enable assay volume scaling when needed

Four assays were conducted simultaneously on the assay plate and results used to determine the mechanism of cytotoxicity. The assays, and measured biomarker, included CellTiter-Glo® (ATP), MultiTox-Fluor (live and dead cell protease activity), Caspase-Glo® 3/7 (caspase 3/7 activity) , and CellTox™ Green (DNA staining).

Initial experiments show that with only minimal optimization, the PIPETMAX 268 is capable of producing reproducible data consistent with toxicity profiles obtained in a manual format (data not shown).