

Add-Mix-Read Assays for the Assessment of Cell Health Using an iPS Cardiomyocyte Cell Model

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1. Abstract

Evaluating cell health is a necessary step in the biobanking process while preparing samples for storage and when propagating cells after cryopreservation. Membrane exclusion dyes are commonly used to evaluate cell viability, but one drawback to this method is the lack of distinction between viable and apoptotic cells with an intact membrane. In addition, the visual scoring of a viable cell is subjective and can vary among users. Homogeneous (add-mix-read) assay reagents are user-friendly alternatives to traditional methods of monitoring cell viability. Reagents have been developed to monitor various aspects of cell health, including viability, cytotoxicity, and apoptosis. The assay reagents can be used individually, or in some cases multiplexed together, to generate a multi-parameter readout of cell health in the same assay well. The process of performing these cell-based assays is simple: reagent is added directly to cells in an assay plate, or to cells in a tube, and incubated for a period of time. Luminescent or fluorescent signal is quantified, depending on the assay. Shown here are data generated with these cell health assays using an iPS cardiomyocyte cell model. Cells were subjected to various treatments in order to elicit an effect for the parameter being measured. Multiplexing assays together conserves cell usage and enables a more complete cell health profile of biorepository samples compared to using a single parameter methodology. Incorporating a quantifiable assay signal removes subjective visual scoring of viable and non-viable cells.

2. Introduction

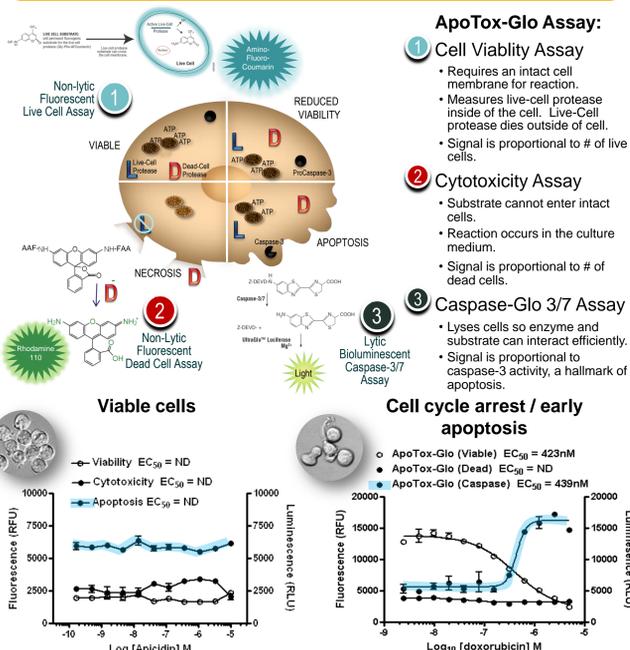
iPS cells are an emerging research tool in a variety of application areas, including toxicity screening. Cardiotoxicity is a lethal side effect of drug treatment, resulting in the removal of therapies from the market due to safety reasons. As a result, screening for off-target effects has become a necessary component of the drug development process.

iCell[®] Cardiomyocytes from Cellular Dynamics are human induced pluripotent stem cell-derived cardiomyocytes. These cells are designed to serve as a cardiac model to aid in drug discovery, and improve predictability of drug efficacy and toxicity screens. As these cells are terminally differentiated human cardiomyocytes, they are expected to generate results that more accurately predict the relevant *in vivo* human response.

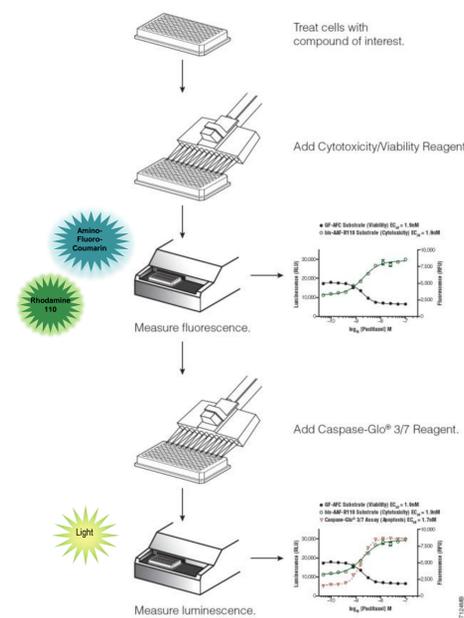
To demonstrate the applicability of iPS-derived cardiomyocytes as an off-target model for toxicity screening, we conducted parallel experiments using a target human K562 cancer cell line model (chronic myelogenous leukemia) alongside the iPS cardiomyocyte cell line. Select drugs known to target cancerous cells were tested, and the toxicity profiles between the two cell types were compared.

To assess overall cell health and cytotoxicity, we used the ApoTox-Glo[™] Triplex Assay, a combination of chemistries used to measure viability (intact cell membrane), cytotoxicity (compromised cell membrane), and apoptosis. This work highlights the use of the triplex assay for drug screening purposes, though the assay is also useful for general cell-health assessment in the absence of drug treatment, with any cell type of interest.

3. The ApoTox-Glo Assay measures viability, cytotoxicity and apoptosis in the same well

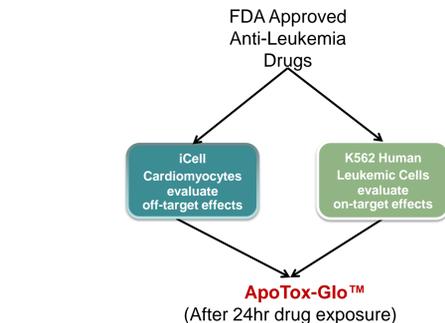
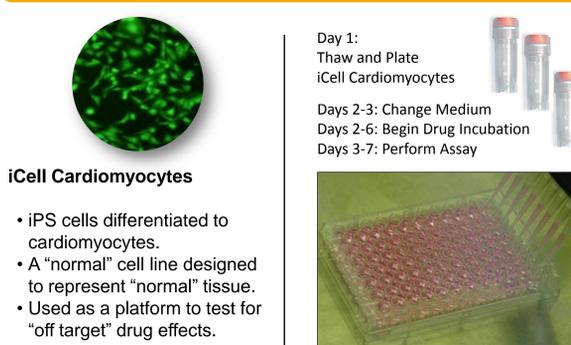


4. Add-mix-read assays are user friendly and easy to incorporate into standard laboratory workflow



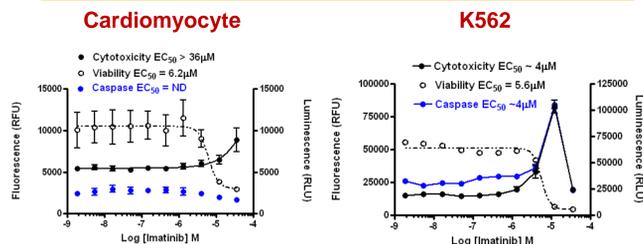
The ApoTox-Glo Assay can be performed in microtubes, or in assay plates as shown here. Reagents are added and mixed to ensure homogeneity. Signal is read with a single-tube or multimode plate reader.

5. iCell Cardiomyocytes are human heart cells derived from induced Pluripotent Stem (iPS) cells



iCell Cardiomyocytes are an effective tool for parallel screening of off-target drug effects that impact cell health.

6. The tyrosine kinase inhibitor Imatinib shows off-target cytotoxic effects in iPS cardiomyocytes

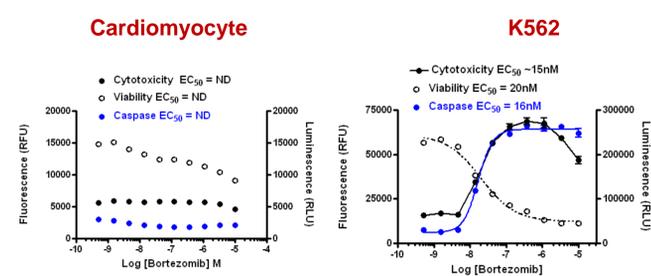


The tyrosine kinase inhibitor Imatinib, brand name Gleevec (Novartis), targets the mutant bcr-abl kinase found in leukocytes of patients with Chronic Myelogenous Leukemia (CML). The immortalized K562 cell line was isolated from a patient with CML, and serves as a model system for the development of cancer therapies targeting this disease.

For all experiments, iPS cardiomyocytes were plated and maintained as previously described. K562s were plated the same day as treatment. Here, a serial titration of Imatinib was applied to each cell line and incubated for 24 hours at 37°C, 5% CO₂. Following treatment, the ApoTox-Glo Assay was performed as previously described.

Cytotoxicity at concentrations greater than 1µM is noted with both cell lines, and implicates potential cardiac toxicity as an off-target effect of imatinib treatment. An increase in caspase-3/7 signal with K562 cells only indicates the mechanism of toxicity to be specific to apoptosis, with general cytotoxicity indicated in cardiomyocytes. Note: diminution of caspase and cytotoxicity biomarker signals at highest doses of imatinib (with K562) are consistent with activation kinetics and time-dependent biomarker decay.

7. The proteasome inhibitor Bortezomib shows cytotoxic target specificity toward K562 but not cardiomyocytes

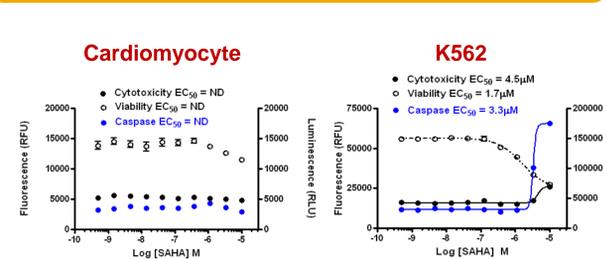


Bortezomib, brand name Velcade (Millenium/Takeda) inhibits the 26S proteasome, part of a complex of proteins thought to be required by cancerous cells to multiply and survive. Bortezomib has been shown to have anti-tumor activity in B cell-type malignancies.

Bortezomib shows potent specificity to K562 and dose dependent effects on cell health, with a decrease in viability and corresponding increase in cytotoxicity. The increase in caspase-3 signal indicates the mechanism of toxicity is due to apoptosis induction. A decrease in cytotoxicity signal at higher concentrations of bortezomib in the K562 model may be a result of activation kinetics and time-dependent biomarker decay. The cardiomyocytes do not show apparent off target effects on cell health*.

*Note: bortezomib, and other proteasome inhibitors, are known to partially inhibit the viability assay protease at concentrations greater than 1 µM, which is over 50-fold the EC₅₀ for K562.

8. The histone deacetylase (HDAC) inhibitor SAHA shows no apparent cytotoxicity in cardiomyocytes



SuberoylAnilide Hydroxamic Acid (SAHA), brand name Vorinostat (Merck) is a cytostatic agent that inhibits tumor cell proliferation by inducing cell cycle arrest and ultimately apoptosis.

SAHA shows less potent specificity to the cancer cell line K562 compared to Bortezomib. Dose dependent effects on cell health, with a decrease in viability and corresponding increase in cytotoxicity at high concentrations, are observed in K562 and not in cardiomyocytes. The increase in caspase-3 signal indicates the mechanism of toxicity is due to apoptosis induction. The cardiomyocytes do not show apparent off target effects on cell health.

9. Conclusions

- The ApoTox-Glo Triplex Assay measures cell viability, cytotoxicity, and apoptosis in the same well.

- The incorporation of three assays in the same well enables the determination of mechanism of action of cell death and can be used to distinguish necrosis from apoptosis-induced cytotoxicity.

- iPS-derived cardiomyocytes are designed to represent normal cardiac tissue and can be used in different applications including screening for off-target cytotoxic drug effects.

- Off-target cytotoxic effects were observed in cardiomyocytes with one of the three drugs used in this study. All three drugs showed apoptosis-inducing cytotoxicity with the K562 cancer target.

- Although drug-induced effects on cell health were demonstrated here, the ApoTox-Glo Assay can be used to assess general cell health of cell lines and primary cells in the absence of treatment. This could enable quantitative assessment of cell health for quality control of biorepository samples.