Abstract
Pathophysiological conditions, medical interventions, and off-target toxicities can all result in cellular oxidative stress. In cardiomyocytes, protected and/or excessive oxidative stress can lead to cardiotoxicity: a primary cause of development delays, black box warnings, and post-launch withdrawal of pharmaceuticals. Many protective responses to oxidative stress are mediated at the transcriptional level through antioxidant response elements (AREs). iCell Cardiomyocytes (Cellular Dynamics International) are fully functional cardiomycocytes derived from human induced pluripotent stem cells (iPScs). The ability to monitor ARE activity in this relevant tissue cell would provide an excellent tool for both interrogating and enhancing cardioprotective processes through basic experimental and screening paradigms, respectively. To that end, a novel ARE-luciferase reporter construct, pGL4[luc2P/NRF2/Hygro] (ARE-luc-Promega) was transiently transfected into iCell Cardiomyocytes and functionally validated by inducing oxidative stress using application of tert-butylhydroperoxide (tBHQ) and monitoring increases in luciferase activity. Control experiments with GFP demonstrated that lipofectamine-based methodologies could introduce the plasmid into the terminally differentiated Cardiomyocytes with greater than 40% transfection efficiency with minimal stimulation with isoproterenol. Together, these data demonstrate that a variety of oxidative stressors could introduce the plasmid into the terminally differentiated Cardiomyocytes with greater than 40% transfection efficiency with minimal stimulation with isoproterenol.

Introduction
Cell Cardiomyocytes are human iPSc-derived cardiomyocytes that recapitulate the biochemical, electrophysiological, mechanical and pathophysiological properties of native human cardiac myocytes. Due to their human origin, high purity, functional relevance, and ease of use, Cardiomyocytes represent an optimal in vitro test system for interrogating cardiac biology in basic research and many areas of drug development.

Aims
• To assess the transcription efficiency of iCell Cardiomyocytes at different times in culture.
• To assess the luciferase reactivity of iCell Cardiomyocytes at different times in culture.
• To assess the induction and suppression of ARE-driven gene expression in iCell Cardiomyocytes.

Methods
Cell Preparation:
Cardiomyocytes were plated in iCell Cardiomyocytes Paking Medium at 20K cells/well in 96-well cell culture plates coated with 0.1% gelatin per manufacturer instructions. Cells were cultured in Cardiomyocytes Maintenance Medium at 37°C, 7% CO2. Medium was changed every 48 hours.

Transfection:
Medium was changed on the day of transfection. Lipofectamine® LTX Plus Reagent was used. Plus reagent and pDNA were combined at a ratio of 1:1 in Opti-MEM® and incubated for 5 minutes at room temperature. Lipofectamine LTX reagent was added to the mixture at a ratio of 2:1. Lipofectamine LTX:DNA and incubated for 30 minutes at room temperature. 25 μg Lipofectamine LTX:A0079 was added to the transfection mixture in 100 μl maintenance medium for a final concentration of 6 μg LTX reagent, 0.2 μg Plus reagent, and 2.5 μg pDNA per well. Cardiomyocytes were transfected at 37°C, 7% CO2 for 18 hours. Medium was changed to a serum-free DMEM-based medium for 6 hours prior to induction.

Luciferase Reporter Vectors:

<table>
<thead>
<tr>
<th>Vector</th>
<th>Description</th>
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<tbody>
<tr>
<td>pGL4[luc2P/NRF2/Hygro] Vector</td>
<td>ARE-luciferase reporter vector</td>
</tr>
<tr>
<td>pGL4[luc2P/MCP/Neo] Vector</td>
<td>CRE-luciferase reporter vector</td>
</tr>
<tr>
<td>pGL4[4x8xLAMp/Neo] Vector</td>
<td>NFAT-luciferase reporter vector</td>
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<tr>
<td>pCMV</td>
<td>CMV promoter reporter vector</td>
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Summary
iCell Cardiomyocytes are human induced pluripotent stem cell-derived cardiomyocytes suitable for a wide variety of applications. Monitoring cell-signaling pathways in a human-based in vitro environment allows for extensive cellular analysis and increased understanding of drug toxicity. iCell Cardiomyocytes are amenable to DNA transfection and exhibit the expected response to the induction and abolition of various cell-signaling pathways. The use of luciferase reporter assays with Cardiomyocytes in preclinical testing provides a means for detecting and validating relevant pathways and targets in drug discovery as well as early detection of toxicity.

GFP transfection efficiency measured in iCell Cardiomyocytes transiently transfected on multiple days in culture. A: iCell Cardiomyocytes transiently transfected with pGL4[luc2P/NRF2/Hygro] vector 12 days after thaw and treated with 1:2 serial dilutions of BHQ for 6 hours. B: iCell Cardiomyocytes transiently transfected with pGL4[luc2P/MCP/Neo] vector 12 days after thaw, and treated with 1:2 serial dilutions of isoproterenol for 6 hours. C: iCell Cardiomyocytes transiently transfected with pGL4[4x8xLAMp/Neo] vector 12 days after thaw, and treated with 1:2 serial dilutions of T3 for 6 hours.

Antioxidants suppress tBHQ-induced ARE-driven gene expression in iCell Cardiomyocytes

A: ARE-Luciferase induction by tBHQ
B: ARE-Luciferase suppression by ascorbic acid
C: ARE-Luciferase suppression by tBHQ

iCell Cardiomyocytes exhibit reporter-driven luciferase activity in response to compound induction.

Luciferase reporter vectors transiently transfected in iCell Cardiomyocytes remain active over prolonged culture durations.

A: CRE 4 days after induction
B: CRE 12 days after induction
C: CRE 21 days after induction

Induction and suppression of ARE-Luciferase activity in iCell Cardiomyocytes

A: ARE-luciferase reporter vector transiently transfected 12 days after thaw with tBHQ and treated with 1:2 serial dilutions of tBHQ for 6 hours. B: ARE-luciferase reporter vector transiently transfected 12 days after thaw with 1:2 serial dilutions of tBHQ for 6 hours. T3-induced ARE-luciferase reporter vector transiently transfected 12 days after thaw with 1:2 serial dilutions of tBHQ for 6 hours.