Techniques for High Throughput Cardiotoxicity Studies Using Human Stem Cell-Derived Cardiomyocytes

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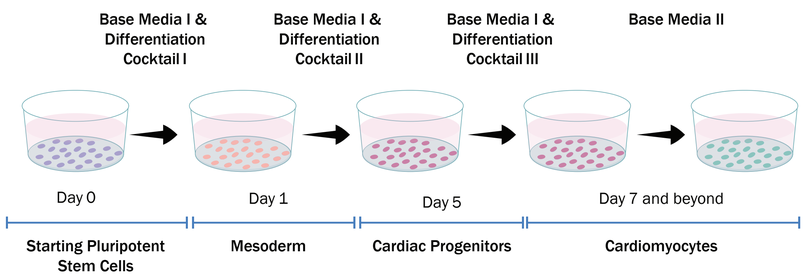
ABSTRACT

Cardiotoxicity is a leading cause for the recall of commercial pharmaceutical drugs and the failure of compounds during drug development. Traditional models for studying cardiotoxicity *in vitro* use non-cardiac cells, such as CHO or HEK cells, transduced with cardiac-muscle related ion channels. These models lack the complexity of *in vivo* cardiomyocytes, thus limiting their predictive power during drug screening. Use of animal models for drug screening is confounded by species differences in ion channels involved in the cardiac action potential. Pluripotent stem cell-derived functional cardiomyocytes provide a simple and renewable alternative model for *in vitro* drug toxicity studies as well as for cardiac disease modeling and the development of clinical therapies. We have previously shown that the StemXVivo™ Cardiomyocyte Differentiation Kit (Catalog # SC032) efficiently differentiates human pluripotent stem cells into functional cardiomyocytes. In this study, we show the utility of the cardiomyocytes produced using the StemXVivo™ Cardiomyocyte Differentiation Kit, including the long-term viability of cardiomyocytes in culture, the successful re-plating of kit-derived cardiomyocytes, and the efficient transfection of cardiomyocytes with fluorescently-tagged wild-type and mutant calcium signaling proteins. We also demonstrate that kit-derived cardiomyocytes respond to known cardio-modulatory small molecules; the α1-adrenoreceptor agonist (Phenylephedrine) increased the rate of cardiomyocyte contraction, while Ca^{2+} channel blockers (Verapamil, Dilatiazem) decreased cardiomyocyte contraction rate. Last, we show that multi-analyte Luminex® technology is an efficient tool for high throughput drug efficacy and toxicity testing. Increases in Pro-BNP secretion and Troponin T release were detected in StemXVivo™ Cardiomyocyte Differentiation Kit-derived cardiomyocyte cultures in response to Endothelin-1 and Doxorubicin treatment, respectively.

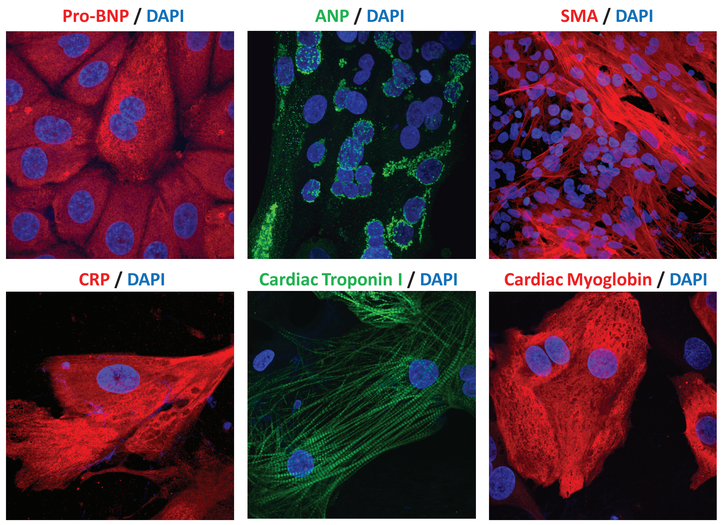
StemXVivo™ Cardiomyocyte Differentiation Kit



Kit Contents

- Cardiomyocyte Differentiation Base Media Supplement I
- Cardiomyocyte Differentiation Base Media Supplement II
- Cardiomyocyte Differentiation Cocktail I
- Cardiomyocyte Differentiation Cocktail II
- Cardiomyocyte Differentiation Cocktail III
- Anti-Human Cardiac Troponin T Antibody

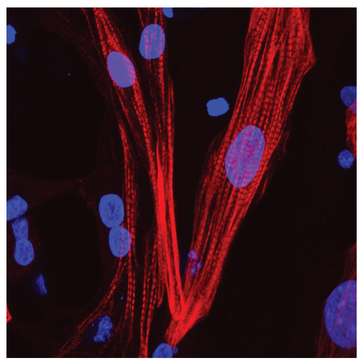
Pluripotent Stem Cell-Derived Cardiomyocytes Express Cell Type-Specific Markers



Detection of cardiomyocyte-specific protein expression by immunocytochemistry. BG01V human embryonic stem cells were differentiated with the StemXVivo™ Cardiomyocyte Differentiation Kit and stained for the following cardiomyocyte-specific markers: Pro-BNP (Brain-type Natriuretic Peptide; Catalog # MAB36041), ANP (Atrial Natriuretic Peptide; Catalog # AF3366), SMA (α-Smooth Muscle Actin; Catalog # MAB1420), CRP (C-Reactive Protein; Catalog # AF1707), Cardiac Troponin I (Catalog # MAB8594), and Cardiac Myoglobin (Catalog # G-125-C). Pro-BNP was visualized with the NorthernLights™ (NL)557-conjugated Goat Anti-Rat Secondary Antibody (Catalog # NL013). ANP was visualized with NL493-conjugated Donkey Anti-Goat Secondary Antibody (Catalog # NL003). SMA was visualized with NL557-conjugated Donkey Anti-Mouse Secondary Antibody (Catalog # NL007). CRP was visualized with NL557-conjugated Donkey Anti-Sheep Secondary Antibody (Catalog # NL010). Cardiac Troponin I was visualized with NL493-conjugated Donkey Anti-Rabbit Secondary Antibody (Catalog # NL006). Cardiac Myoglobin was visualized with NL557-conjugated Donkey Anti-Goat Secondary Antibody (Catalog # NL001).

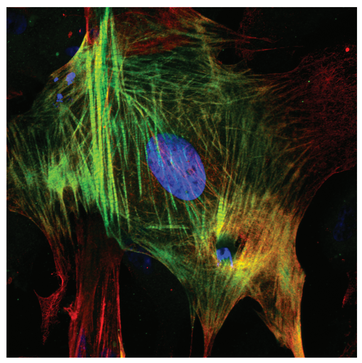
Pluripotent Stem Cell-Derived Cardiomyocytes Offer Experimental Flexibility

Maintain Healthy ESC-Derived Cardiomyocytes in Long-Term Cultures



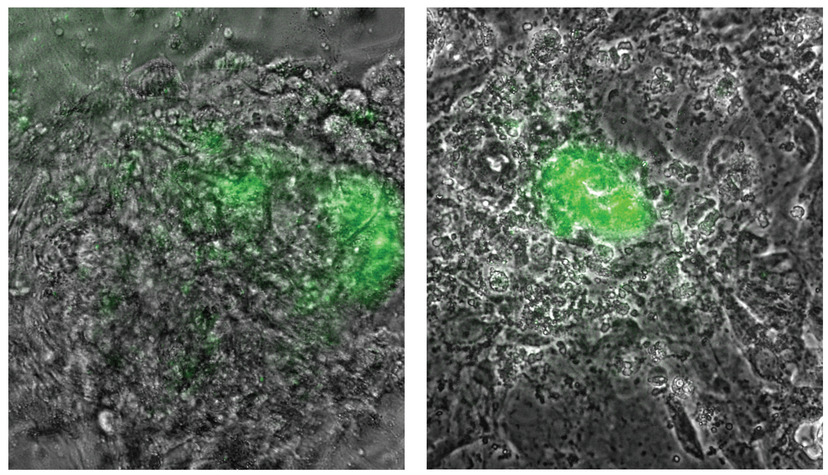
Extended culture of pluripotent stem cell-derived cardiomyocytes. BG01V human embryonic stem cell-derived cardiomyocytes were maintained in StemXVivo™ Cardiomyocyte Maintenance Media Supplement (Catalog # AR011) for 80 days. Cells maintained in long-term culture exhibited continued expression of Cardiac Troponin I (red; Catalog # MAB8594). Cardiac Troponin I was visualized with the NL557-conjugated Donkey Anti-Rabbit Secondary Antibody (Catalog # NL004). Cells were counterstained with DAPI (blue).

Split and Re-Plate ESC-Derived Cardiomyocytes to Expand Experimental Flexibility



Replating of pluripotent stem cell-derived cardiomyocytes onto fibronectin-coated plates. BG01V human embryonic stem cell-derived cardiomyocytes were dissociated using Accutase™, suspended in StemXVivo™ Cardiomyocyte Maintenance Media Supplement (Catalog # AR011) containing 20% FBS, and re-plated into plates coated with fibronectin (Catalog # 1918-FN). Re-plated cells re-initiated rhythmic contractions within 5–7 days and maintained expression of the cardiomyocyte-specific markers, Cardiac Troponin I (green; Catalog # MAB8594) and Cardiac Troponin T (red; Catalog # MAB1874). Cells were counterstained with DAPI (blue).

Transfect ESC-derived Cardiomyocytes to Study Cell Function and Intracellular Signaling



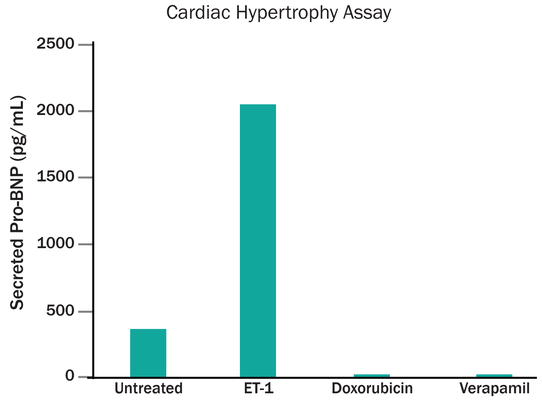
Transfection of pluripotent stem cell-derived cardiomyocytes. BG01V human embryonic stem cell-derived cardiomyocytes were transfected with GFP-tagged wild-type Calmodulin (Calmodulin-GFP) or mutant Calmodulin (D130G-Calmodulin-GFP) using ViaFect™ Transfection Reagent (Promega). Transfection was confirmed by visualization of GFP expression. Images show GFP (green) expression in Calmodulin-GFP and D130G-Calmodulin-GFP transfected cardiomyocytes. Fluorescent images are overlaid onto bright field images.

Drug Efficacy Screening Using Pluripotent Stem Cell-Derived Cardiomyocytes

Small molecules affect cardiomyocyte contraction rates. The effect of small molecules on cardiomyocyte contraction rate was assessed by monitoring intracellular calcium fluctuations, where an increase in calcium coincides with cardiomyocyte contraction. StemXVivo™ Kit-differentiated cardiomyocytes were loaded with the fluorescent calcium indicator, Fluo-4, prior to small molecule treatment. Individual wells were first monitored for baseline rates of calcium fluctuation. represented as beats per minute (bpm). **A**) Phenylephedrine (3uM, Catalog # 2838), an α1-adrenoceptor agonist, increased the calcium fluctuation rate of cardiomyocytes compared to untreated cells. **B**) Diltiazem (30uM, Catalog # 0685), a L-type calcium channel blocker, decreased the rate of calcium fluctuations in cardiomyocytes compared to untreated cells. **C**) Verapamil (10uM or 30uM, Catalog # 0654), another L-type calcium channel blocker, decreased the rate of calcium fluctuations in cardiomyocytes in a dose-dependent manner.

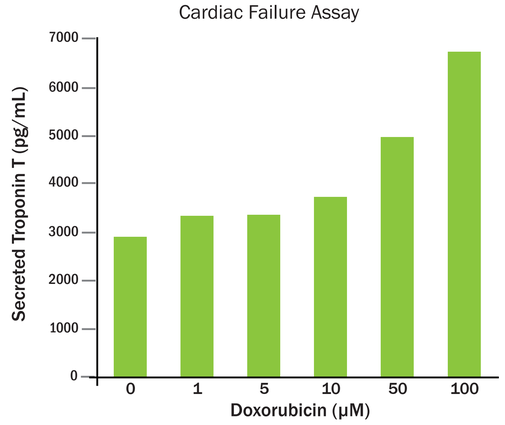
High Throughput Screening for Cardiotoxicity

Cardiac Hypertrophy Assay



Pluripotent stem cell-derived cardiomyocytes secrete Pro-BNP in response to small molecule-induction of cardiac hypertrophy. Cardiomyocytes were treated with the vasoconstrictor, ET-1 (Endothelin-1; Catalog # 1160), or one of two L-type calcium channel inhibitors, Doxorubicin (Catalog # 2252) or Verapamil (Catalog # 0654). Levels of the cardiac hypertrophy marker, Pro-Brain Natriuretic Peptide (Pro-BNP), were detected via Luminex® bead-based multiplex assay (Catalog # LXSAH). A substantial increase in Pro-BNP was detected in the medium of cardiomyocytes treated with ET-1, a known inducer of cardiac hypertrophy. Conversely, Doxorubicin and Verapamil decreased Pro-BNP secretion from cardiomyocytes.

Cardiac Failure Assay



Doxorubicin induces secretion of Troponin T from pluripotent stem cell-derived cardiomyocytes. Cardiomyocytes were treated with increasing doses of Doxorubicin (Catalog # 2252) for 24 hours. High concentration or extended exposure to Doxorubicin is known to induce heart failure. Doxorubicin-induced cardiac failure was quantified by measuring secreted Troponin T, a known biomarker of heart failure. Troponin T was detected via Luminex® bead-based multiplex assay (Catalog # LXSAH). Increased levels of secreted Troponin T are observed with increasing concentrations of Doxorubicin.

CONCLUSIONS

- The StemXVivo™ Cardiomyocyte Differentiation Kit efficiently differentiates human pluripotent stem cells into cardiomyocytes.
- Cells generated using the StemXVivo™ Cardiomyocyte Differentiation Kit express cardiomyocyte-specific markers.
- Cardiomyocytes can be re-plated and maintained in culture over extended periods of time.
- Cardiomyocytes can be transfected to study the effects of specific proteins on cardiomyocyte function.
- Cardiomyocytes can be used for assessment of cardiac drug efficacy and toxicity.
- Multi-analyte Luminex® technology can be combined with pluripotent stem cell-derived cardiomyocytes for high throughput small molecule and drug screening.

BG01V human embryonic stem cells are licensed from ViaCyte, Inc.