EFFICIENT AND SCALABLE GMP-GRADE CULTURE MEDIA SYSTEM FOR RAPID CARDIOMYOCYTE DIFFERENTIATION OF PLURIPOTENT STEM CELLS IN HUMAN DISEASE RESEARCH

life technologies
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ABSTRACT

Introduction: Simple and robust derivation of spontaneously contracting cardiomyocytes derived from human pluripotent stem cells (hPSCs) would provide a valuable source of cells for basic research into cardiac biology and mechanisms of heart disease as well as applied studies in pharmacological drug discovery and toxicity screening. Currently a number of protocols exist for inducing embryoid bodies (EB) suspension or monolayer cultures of hPSC to differentiate into cardiomyocyte (1, 2). These cardiomyocyte differentiation protocols have led to varying results and differing purity levels of cardiomyocytes. To enable consistent differentiation of hPSCs, we developed a simplified cardiomyocyte differentiation media system, consisting of three ready-to-use components. This easy to use cardiac differentiation system is designed for monolayer hPSC affording flexible culture formats and a scalable workflow enabling generation of large numbers of consistent, spontaneously active cardiomyocytes.

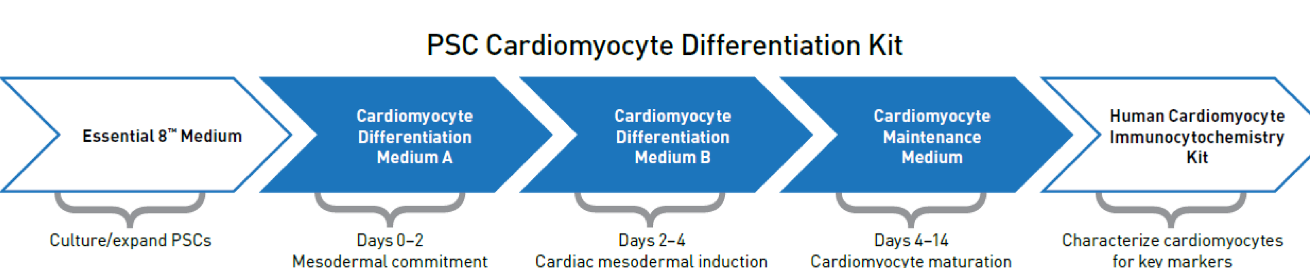
Methods: Briefly, hPSCs were maintained under feeder-free conditions using Essential 8 Medium. For cardiac induction EDTA-dissociated cells were seeded on Geltrex-coated surface as small clusters at ~1 - 2 x 10^4/cm^2 by day 4 or 5 under serum-free condition. After reaching target confluence (Day 0), hPSC were incubated in an induction media for two days followed by addition of a second induction media and cultured for two additional days. In the final step, the induction media was replaced with maintenance media and cells were re-fed every other day for up to five weeks with beating cells usually first appearing on Day 7 and robust contracting syncytium by Day 10.The differentiated cells were analyzed for formation of contracting syncytium and for cardiomyocyte markers by flow cytometry and immunocytochemistry.

Results: Multiple parameters were evaluated during differentiation media testing and our results demonstrate significant influences arise from the quality and confluence level of the PSC prior to induction and with the incubation period in different media. For example, several studies showed that hPSCs seeded at a density to reach 70 to 85% confluency by four days would yield large numbers of synchronously beating cardiomyocytes. When analyzed by quantitative flow cytometry, we observed troponin T type 2 (TNNT2)-immunoreactive cells strongly correlating to contracting cardiomyocytes. In cultures initiated with 90% or greater confluency, we typically observed lower yields of correlated TNNT2+ contracting cardiomyocytes. Subsequent immunocytochemistry studies verified expression of cardiac markers of ISL1, GATA4, MEF2C and MYH6 in Day 15 cardiomyocyte cultures.

Conclusions: Our findings suggest the complete differentiation media system could serve as a standardized culture system for generating large numbers of consistent, spontaneously active cardiomyocytes in research studies. Further investigations will be performed to assess cardiomyocyte differentiation on different hPSC lines as well as verifying gene expression profiles, cardiac markers, and sensitivity to pharmacological agents.

INTRODUCTION

Figure 1. New Human PSC Differentiation Kit



PSC Cardiomyocyte Differentiation Kit

SKU: A25042SA

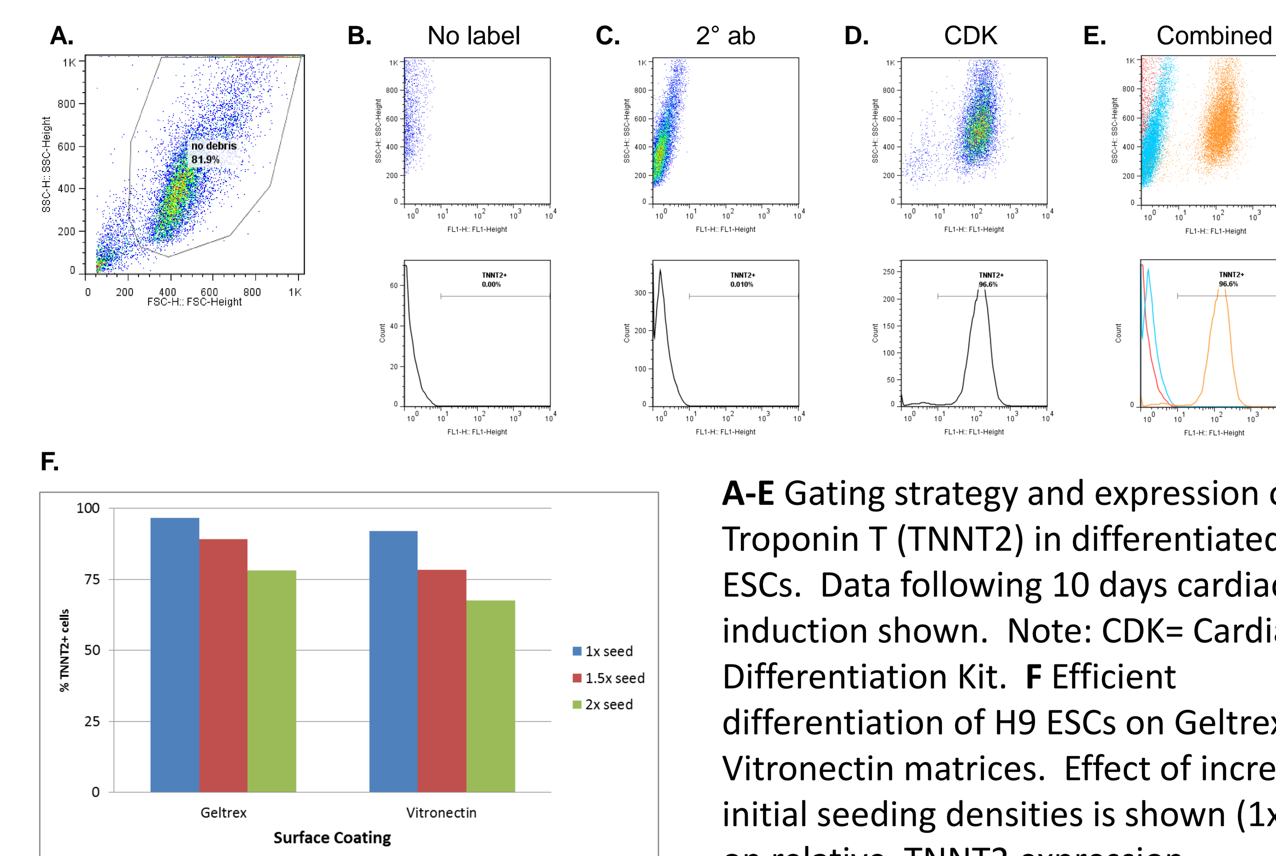
Kit contains enough medium for eight 12-well plates

- System comprised of three 1x (ready to use) media used in succession
- Capable of producing contracting cardiomyocytes in as few as 8 days from hPSCs
- Ships at ambient temperature, stored at 2-8 ºC (no frozen storage required)
- Cardiomyocytes can be maintained in culture for >28 days
- Maintenance medium also sold separately for long term culture

Timepoint	Day -4	Day 0	Day 6	Day 8	Day 10	Day 12
No. cells (x10e6/well)	0.054±0.004	1.59±0.11	6.58±0.20	5.64±0.56	5.46±0.15	5.43±0.15
Fold Yield	1	29.6	122.4	104.9	101.5	101.0

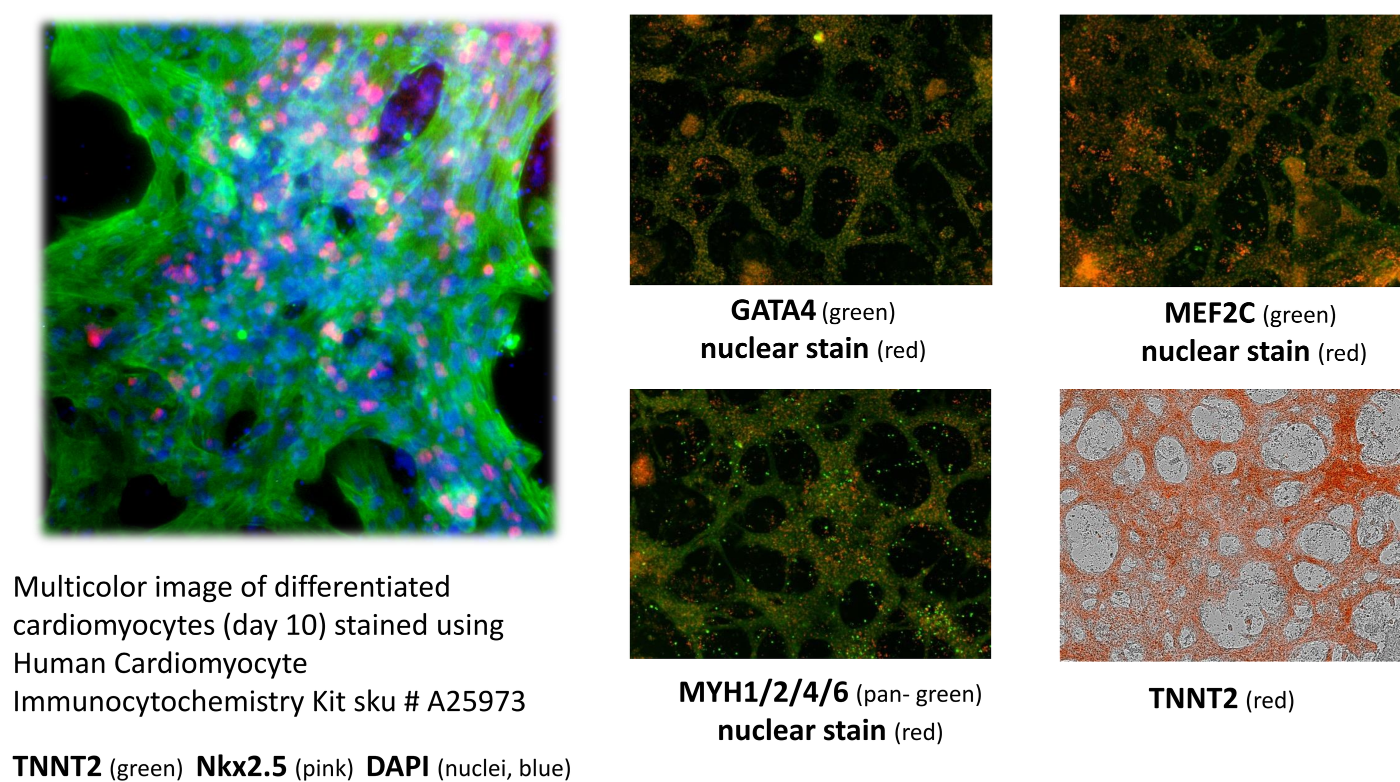
RESULTS

Figure 2. Flow Cytometry Analysis for Troponin T Expression in Differentiated Cardiomyoctyes



A-E Gating strategy and expression of Troponin T (TNNT2) in differentiated H9 ESCs. Data following 10 days cardiac induction shown. Note: CDK= Cardiac Differentiation Kit. **F** Efficient differentiation of H9 ESCs on Geltrex™ or Vitronectin matrices. Effect of increased initial seeding densities is shown (1x-2x) on relative TNNT2 expression

Figure 3. Immunocytochemical Staining of Differentiated Cardiomyocytes



GATA4 (green) **nuclear stain** (red)

MEF2C (green) **nuclear stain** (red)

MYH1/2/4/6 (pan- green) **nuclear stain** (red)

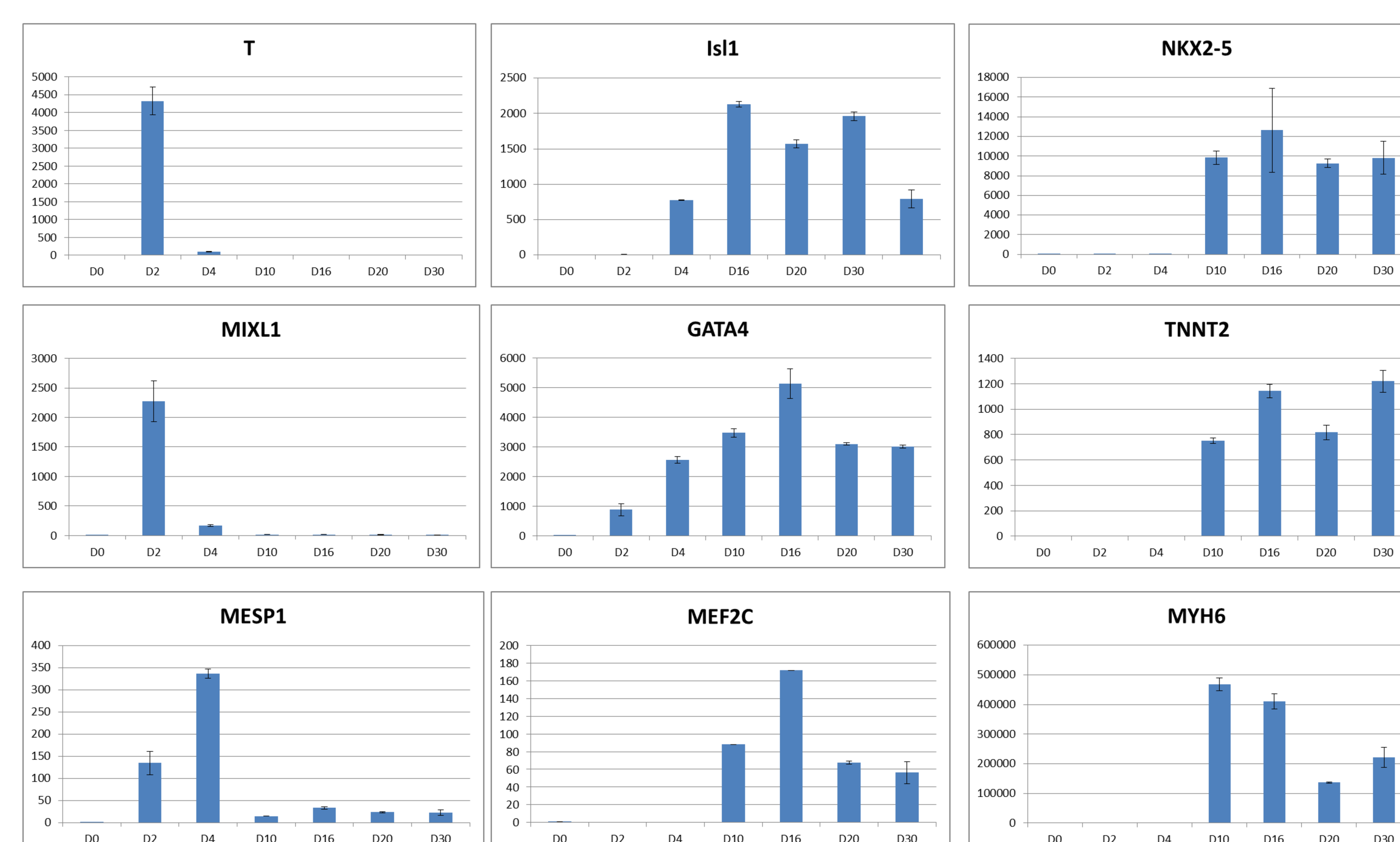
TNNT2 (red)

Multicolor image of differentiated cardiomyocytes (day 10) stained using Human Cardiomyocyte Immunocytochemistry Kit sku # A25973

TNNT2 (green) **Nkx2.5** (pink) **DAPI** (nuclei, blue)

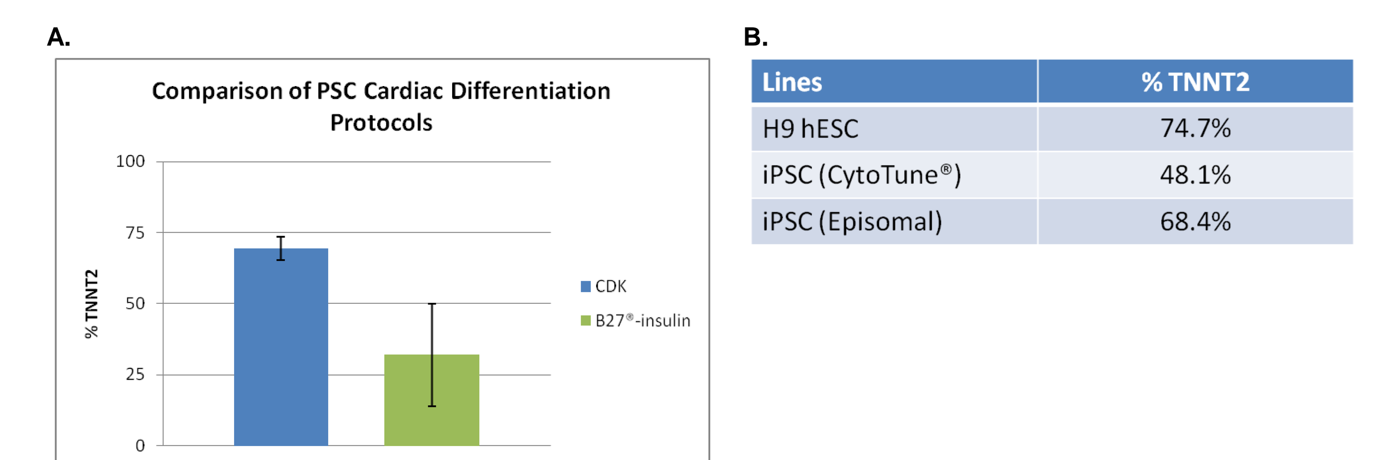
Figure 4. Time Course Gene Expression Analysis of PSC Differentiation to Cardiomyocytes

Mesoderm Commitment (D0-D3)	Early Cardiomyocyte (D4-D6)	Late Cardiomyocyte (D7-D15)
T (brachyury)	ISL1	NKX2-5
MIXL1	GATA4	TNNT2
MESP1	MEF2C	MYH6



TaqMan® qPCR analysis of differentiated (H9) ESCs from time of induction (day 0) through out day 30. Relative gene expression for each time point is shown, normalized to day 0 expression. Data is presented as relative fold change compared to day 0 control samples.

Figure 5. PSC Cardiac Differentiation Kit: Comparison of Methods and Sources of Cells

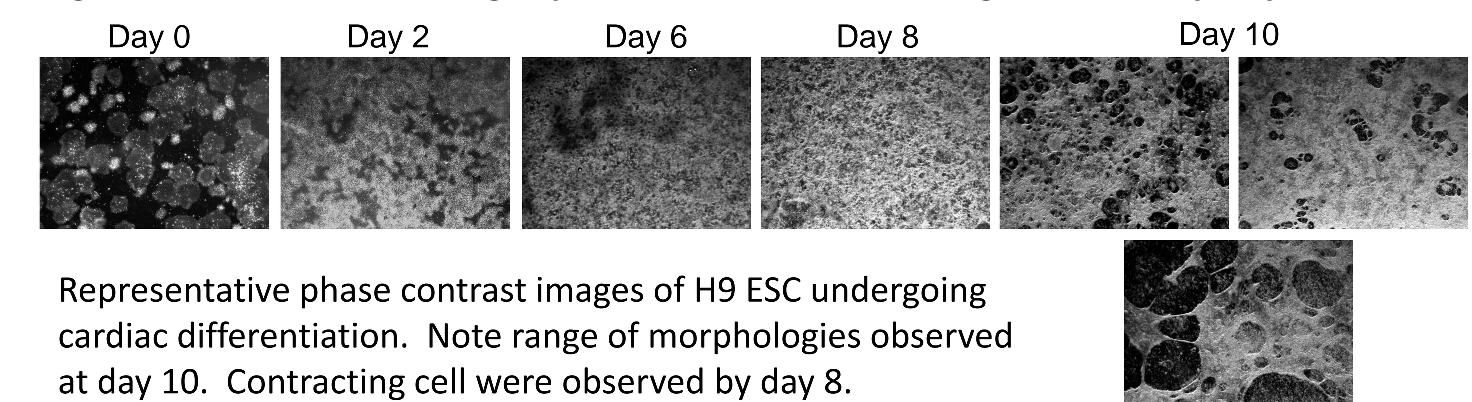


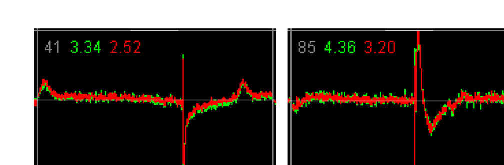
B.

Lines	% TNNT2
H9 hESC	74.7%
iPSC (CytoTune®)	48.1%
iPSC (Episomal)	68.4%

A. Cardiac differentiation of H9 ESCs for 17 days. Flow cytometry for TNNT2 demonstrates our new PSC Cardiac Differentiation Kit (CDK) out performs the "homebrew" protocol based on B27® minus insulin protocol (3).
B. Comparison of PSC Cardiac Differentiation Kit across different PSC lines (indicated). TNNT2 expression following 10-11 days cardiac induction is shown.

Figure 6. Photomicrographs of Differentiating Cardiomyocytes





Representative phase contrast images of H9 ESC undergoing cardiac differentiation. Note range of morphologies observed at day 10. Contracting cell were observed by day 8.

Figure 7. Differentiated Cardiomyocytes Demonstrate Spontaneous Electrical Activity and Contraction

Representative Multi Electro Array (MEA) recordings of stem cell (H9) derived cardiomyocyte electrophysiology. Cells were differentiated 16 days, cryopreserved then replated and incubation in Cardiac Maintenance Media (CMM) for 5 days prior to recording.

CONCLUSIONS

- We have launched a simple and robust 3 part media system for differentiation of human PSCs to contracting cardiomyocytes
- The media is provided as a complete system -no supplementation required
- Electrically coupled contracting syncitia is observed as early as 8 days following cardiac differentiation.
- Both iPSC and ES lines have been shown to efficiently differentiate
- iPSC derived using episomal and Sendai virus (CytoTune®) methods have been successfully differentiated

REFERENCES

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ACKNOWLEDGEMENTS

Authors thank their colleagues for significant project support , including Rhonda Newman, Stacy Jones, Lauren Sangenario, Mike Hancock and Marian Piekarczyk.

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