

Efficient generation of cardiomyocytes from human pluripotent stem cells

Background

The advancement of methods for the efficient generation of cardiac cells from human pluripotent stem cells (hPSCs) is of great interest for cardiovascular disease modeling, drug safety studies, and development of cell replacement strategies. Various differentiation protocols have been developed, which either rely on the formation of embryoid bodies (EB) in suspension culture or on monolayer-based protocols. More recently, it was shown that hPSCs can be efficiently differentiated into cardiomyocytes by a temporal modulation of Wnt/ β -catenin signaling using small molecules¹⁻³. This approach is particularly compelling since it allows the robust generation of cardiomyocytes under highly defined cell culture conditions.

Materials

- StemMACS™ iPS-Brew XF, human (# 130-104-368)
- TrypLE™ Select Enzyme (1x), no phenol red, liquid (Life Technologies®)
- Soybean Trypsin Inhibitor, powder (Life Technologies)
- StemMACS Thiazovivin (# 130-104-461)
- RPMI 1640 + L-Glutamine (Life Technologies)
- B-27® Supplement (Life Technologies)
- B-27 Supplement, minus insulin (Life Technologies)
- StemMACS CHIR99021 (# 130-103-926)
- IWR-1 (Sigma-Aldrich®)
- 12-well plates coated with Matrigel® hESC-Qualified Matrix (Corning®)
- Dulbecco's phosphate-buffered saline (DPBS) without Ca^{2+} and Mg^{2+} (e.g. Lonza, #BE17-512F)

Additional requirements for flow cytometric analysis

- MACSQuant® Analyzer 10 (# 130-096-343)
- Fluorochrome-conjugated antibodies, e.g., Anti-Cardiac Troponin T-FITC (# 130-106-687), Anti- α -Actinin (Sarcomeric)-FITC (# 130-106-936), Anti-Myosin Heavy Chain-APC (# 130-106-215), Anti-MLC2a-APC (# 130-106-143), and Anti-MLC2v-PE (# 130-106-133).

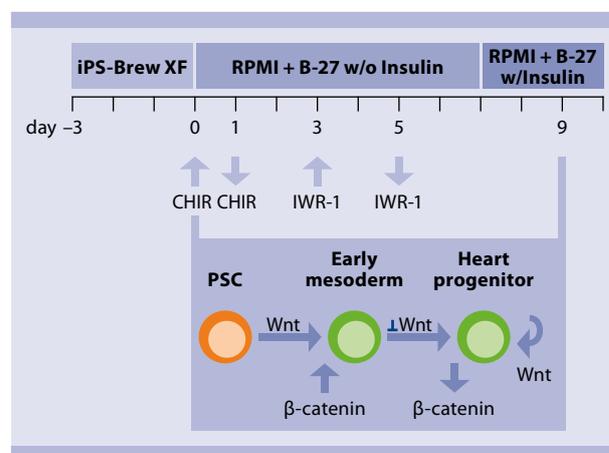


Figure 1: Outline of the differentiation protocol: hPSCs are maintained under xeno-free conditions in StemMACS iPS-Brew XF medium and differentiated into cardiomyocytes using a monolayer protocol^{1,2} with consecutive activation (CHIR99021) and inhibition (IWR-1) of Wnt signaling.

Differentiation protocol

Note: Testing of various cell numbers and CHIR99021 concentrations is recommended to optimize differentiation for each individual hPSC line. See figure 1 for a graphical overview of the procedure.

Day -3: Cell seeding

1. Harvest hPSCs at 90% confluence as follows:
 - a. Remove culture medium and wash cells once with DPBS w/o Ca^{2+} and Mg^{2+} .
 - b. Add 1 mL TrypLE Select Enzyme per well (6-well plate).
 - c. Incubate for 5 minutes at 37 °C.
 - d. Stop enzymatic reaction by adding 1 mL/well of Soybean Trypsin Inhibitor.
 - e. Dissociate cell layer to obtain a single-cell suspension by pipetting up and down using a 5 mL serological pipette.
2. Determine the cell number using the MACSQuant® Analyzer 10.
3. Centrifuge cell suspension at 125xg for 5 minutes to collect cells. Aspirate supernatant completely.

- Adjust cell densities (1.5×10^5 to 7×10^5 cells/mL) in StemMACS iPS-Brew XF + 2 μ M StemMACS Thiazovivin.
- Seed cells into a Matrigel Matrix–coated 12-well plate with a final volume of 1 mL per well.

Note: The effectiveness of differentiation depends on the number of seeded cells. Therefore, it is crucial to determine the cell number that leads to optimal differentiation. Different cell numbers might be required for different hPSC lines.

Day –2/–1: Medium exchange

- Replace medium with 1 mL StemMACS iPS-Brew XF per well.

Day 0: Adjustment of CHIR99021 concentration

- Prepare medium RPMI 1640 + L-Glutamine + B-27 Supplement, minus insulin with different CHIR99021 concentrations (8–12 μ M).
- Replace medium with 1 mL of the prepared CHIR99021-containing medium per well.

Note: Cells should be confluent at this point. Incubate with CHIR99021 for exactly 24 hours.

Day 1: Medium exchange

- Replace medium with 2 mL of RPMI 1640 + L-Glutamine + B-27 Supplement, minus insulin per well.

Day 3: Addition of IWR-1

- Prepare conditioned medium as follows:
 - Remove 1 mL/well of the old cell culture medium and transfer it into a tube.
 - Add 1 mL/well fresh RPMI 1640 + L-Glutamine + B-27 Supplement, minus insulin into the tube.
 - Add 5 μ M IWR-1 into the tube.
- Replace medium with 2 mL of the conditioned medium per well.

Day 5: Medium exchange

- Replace medium with 2 mL of RPMI 1640 + L-Glutamine + B-27 Supplement, minus insulin per well.

Day 7: Medium exchange

- Replace medium with 2 mL of RPMI 1640 + L-Glutamine + B-27 Supplement w/Insulin per well.
- Replace medium with 2 mL of RPMI 1640 + L-Glutamine + B-27 Supplement w/Insulin every 2–3 days per well.

Results

This protocol enables the generation of cardiomyocytes from hPSCs with efficacies of up to 80%, giving rise to contracting cells after 9 to 12 days. Figure 2 shows a typical result: The differentiated cells displayed a high expression level of the cardiomyocyte-specific marker troponin T as shown by flow cytometry. The use of recombinantly engineered REAfinity™ Antibodies against cardiac troponin T, α -actinin, or other cardiomyocyte specific markers like myosin heavy chain, MLC2a, and MLC2v allow a detailed analysis of cardiomyocytes and respective subtypes. Immunofluorescence microscopy revealed the typical cardiomyocyte morphology and sarcomeric structure.

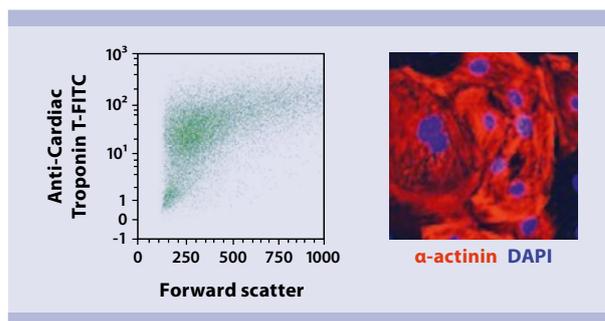


Figure 2: Cardiomyocytes differentiated from hPSCs: Cells were analyzed by flow cytometry and immunofluorescence microscopy.

References

- Lian, X. *et al.* (2012) Cozzarelli Prize Winner: Robust cardiomyocyte differentiation from human pluripotent stem cells via temporal modulation of canonical Wnt signaling. *Proc. Natl. Acad. Sci. U.S.A.* 109: E1848–E1857.
- Lian, X. *et al.* (2013) Directed cardiomyocyte differentiation from human pluripotent stem cells by modulating Wnt/ β -catenin signaling under fully defined conditions. *Nat. Protoc.* 8: 162–175.
- Burridge, P.W. *et al.* (2014) Chemically defined generation of human cardiomyocytes. *Nat. Methods* 11: 855–860.

MACS® Product	Order no.
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Miltenyi Biotec

Miltenyi Biotec GmbH | Friedrich-Ebert-Straße 68 | 51429 Bergisch Gladbach | Germany | Phone +49 2204 8306-0 | Fax +49 2204 85197
 macs@miltenyibiotec.de | www.miltenyibiotec.com

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