STEMdiff™ Atrial Cardiomyocyte Differentiation Kit

Culture medium kit for differentiation of human PSCs to atrial cardiomyocytes

Catalog #100-0215 1 Kit

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Product Description

STEMdiff™ Atrial Cardiomyocyte Differentiation Kit includes a medium for differentiation of human embryonic stem cells (hESCs) and human induced pluripotent stem cells (hiPSCs; e.g. Healthy Control Human iPSC Line, Female, SCTi003-A, Catalog #200-0511) into atrial cardiomyocytes (cardiac troponin T-positive [cTnT+]), as well as a medium for maintenance of hPSC-derived atrial cardiomyocytes. This kit can be used to generate atrial cardiomyocytes derived from a clump culture of hPSCs maintained in mTeSR™1 (Catalog #85850), mTeSR™ Plus (Catalog #100-0276), or TeSR™-E8™ (Catalog #05990). Greater than 80% of these cells will be cTnT+. An average of 1 x 10^6 cells can be harvested from a single well of a 12-well plate.

STEMdiff™ Cardiomyocyte Maintenance Kit (Catalog #05020) comprises the maintenance basal medium and supplement; it can be used for long-term maintenance of hPSC-derived cardiomyocytes for one month or longer. These cardiomyocytes can be used in various downstream applications and analyses.

Product Information

The following components are sold as a complete kit (Catalog #100-0215) and are not available for individual sale.

COMPONENT NAME	COMPONENT #	SIZE	STORAGE	SHELF LIFE
STEMdiff™ Cardiomyocyte Differentiation Basal Medium	05011	380 mL	Store at 2 - 8°C.	Stable for 12 months from date of manufacture (MFG) on label.
STEMdiff™ Atrial Cardiomyocyte Differentiation Supplement A (10X)*	100-0216	10 mL	Store at -20°C.	Stable for 2 years from date of manufacture (MFG) on label.
STEMdiff™ Atrial Cardiomyocyte Differentiation Supplement B (10X)*	100-0217	10 mL	Store at -20°C.	Stable for 2 years from date of manufacture (MFG) on label.
STEMdiff™ Atrial Cardiomyocyte Differentiation Supplement C (10X)*	100-0218	20 mL	Store at -20°C.	Stable for 2 years from date of manufacture (MFG) on label.
STEMdiff™ Cardiomyocyte Maintenance Basal Medium†	05015	490 mL	Store at 2 - 8°C.	Stable for 12 months from date of manufacture (MFG) on label.
STEMdiff™ Cardiomyocyte Maintenance Supplement (50X)*†	05016	10 mL	Store at -20°C.	Stable for 2 years from date of manufacture (MFG) on label.

*This component contains material derived from human plasma. Donors have been tested and found negative for HIV-1 and -2, hepatitis B, and hepatitis C prior to donation. However, this product should be considered potentially infectious and treated in accordance with universal handling precautions.
†Also available as part of STEMdiff™ Cardiomyocyte Maintenance Kit (Catalog #05020).

Materials Required but Not Included

PRODUCT NAME	CATALOG #
Corning® Matrigel® hESC-Qualified Matrix	Corning 354277
D-PBS (Without Ca++ and Mg++)	37350
Gentle Cell Dissociation Reagent	100-0485
Hausser Scientific™ Bright-Line Hemocytometer	100-1181
mTeSR™1 OR mTeSR™ Plus OR TeSR™-E8™	85850 OR 100-0276 OR 05990
Trypan Blue	07050
Y-27632	72302

Preparation of Media

A. PREPARATION OF STEMdiff™ ATRIAL CARDIOMYOCYTE DIFFERENTIATION MEDIUM A, B, & C (MEDIUM A, B, & C)

Use sterile technique to prepare Medium A, B, or C (STEMdiff™ Cardiomyocyte Differentiation Basal Medium + STEMdiff™ Atrial Cardiomyocyte Differentiation Supplement A, B, or C [10X]). The following example is for preparing 100 mL of Medium A. If preparing other volumes, adjust accordingly. For Medium B and Medium C, follow the instructions below, replacing STEMdiff™ Atrial Cardiomyocyte Differentiation Supplement A (10X) with STEMdiff™ Atrial Cardiomyocyte Differentiation Supplement B (10X) or STEMdiff™ Atrial Cardiomyocyte Differentiation Supplement C (10X), respectively.

1. Thaw STEMdiff™ Atrial Cardiomyocyte Differentiation Supplement A (10X) at room temperature (15 - 25°C). Mix thoroughly.

   NOTE: If not used immediately, aliquot Supplement and store at -20°C. Do not exceed the shelf life of the Supplement. Once aliquots are thawed, do not re-freeze.

2. Add 10 mL of STEMdiff™ Atrial Cardiomyocyte Differentiation Supplement A (10X) to 90 mL of STEMdiff™ Cardiomyocyte Differentiation Basal Medium. Mix thoroughly.

   NOTE: If not used immediately, store Medium A, B, or C at 2 - 8°C for up to 2 weeks.
   Warm medium to room temperature before use.

B. PREPARATION OF STEMdiff™ CARDIOMYOCYTE MAINTENANCE MEDIUM

Use sterile technique to prepare STEMdiff™ Cardiomyocyte Maintenance Medium (STEMdiff™ Cardiomyocyte Maintenance Basal Medium + STEMdiff™ Cardiomyocyte Maintenance Supplement [50X]). The following example is for preparing 500 mL of complete medium. If preparing other volumes, adjust accordingly.

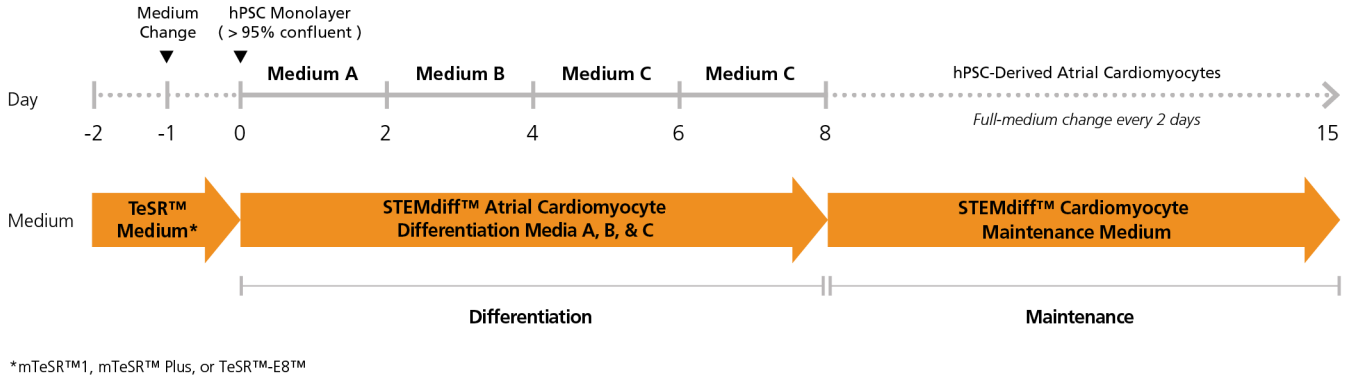
1. Thaw STEMdiff™ Cardiomyocyte Maintenance Supplement (50X) at room temperature (15 - 25°C). Mix thoroughly.

   NOTE: If not used immediately, aliquot Supplement and store at -20°C. Do not exceed the shelf life of the Supplement. Once aliquots are thawed, do not re-freeze.

2. Add 10 mL of STEMdiff™ Cardiomyocyte Maintenance Supplement (50X) to 490 mL of STEMdiff™ Cardiomyocyte Maintenance Basal Medium. Mix thoroughly.

   NOTE: If not used immediately, store STEMdiff™ Cardiomyocyte Maintenance Medium at 2 - 8°C for up to 4 weeks. Warm medium to room temperature before use.

Protocol Diagram



*mTeSR™1, mTeSR™ Plus, or TeSR™-E8™

Figure 1. Protocol diagram for hPSC Differentiation to Atrial Cardiomyocytes

Directions for Use

Please read the entire protocol before proceeding. Use sterile technique when performing the following protocols.

A. DISSOCIATION OF hPSCs INTO A SINGLE-CELL SUSPENSION

Start with a clump culture of hPSCs maintained in mTeSR™1, mTeSR™ Plus, or TeSR™-E8™ on Corning® Matrigel®-coated 6-well plates. It is critical to start with high-quality hPSC cultures for efficient cardiomyocyte differentiation. hPSCs must have high expression of pluripotency markers, e.g. OCT4 and TRA-1-60.

For complete instructions on maintaining hPSCs in TeSR™ media, and for coating plates with Corning® Matrigel®, refer to the Technical Manual for mTeSR™1, mTeSR™ Plus, or TeSR™-E8™, available at www.stemcell.com, or contact us to request a copy.

1. Coat a 12-well tissue culture plate with Corning® Matrigel® and bring to room temperature (15 - 25°C) for at least 1 hour prior to use.
2. Wash each well to be passaged with 1 mL of D-PBS (Without Ca++ and Mg++).
3. Aspirate the wash and add 1 mL/well of Gentle Cell Dissociation Reagent.
4. Incubate at 37°C and 5% CO_2 for 8 - 10 minutes.
5. In each well, dislodge cells by pipetting up and down 3 - 4 times using a pipettor with a 1000 µL tip.
6. Immediately transfer cells to a tube containing 1 mL of mTeSR™1, mTeSR™ Plus, or TeSR™-E8™ per well harvested.
7. Centrifuge at 300 x g for 5 minutes. Remove and discard supernatant.
8. Gently resuspend cell pellet with 1 - 2 mL of mTeSR™1, mTeSR™ Plus, or TeSR™-E8™ supplemented with 10 µM Y-27632.
9. Perform a cell count using Trypan Blue and a hemocytometer.
10. Proceed to section B for culture of single-cell hPSCs.

B. CULTURE OF SINGLE-CELL hPSCs

1. Day -2: Aspirate Corning® Matrigel® from a coated 12-well plate (prepared in section A, step 1). Add 1 mL of mTeSR™1, mTeSR™ Plus, or TeSR™-E8™ supplemented with 10 µM Y-27632 per well.
2. Add hPSCs (from section A) at a density of 3.5 - 8 x 10^5 cells/well. Move the plate in several quick, short, back-and-forth and side-to-side motions to ensure uniform distribution of cells.

   NOTE: A range of seeding densities is provided to account for differences in hPSC lines and variations in their rate of proliferation during maintenance culture. Cells must reach > 95% confluency after 48 hours of incubation (steps 3 - 4) and before starting the differentiation protocol (section C).
3. Incubate at 37°C for 24 hours. Do not disturb cells.
4. Day -1: Remove medium and replace with 1 mL of fresh mTeSR™1, mTeSR™ Plus, or TeSR™-E8™ (without Y-27632). Incubate at 37°C for 24 hours. Do not disturb cells.
5. Assess cells for confluency.

   CRITICAL: Cells must reach > 95% confluency before starting the differentiation protocol (section C). Figure 2 is a representative example of this level of confluency. If cells are < 95% confluent, do not continue incubation. Instead, repeat steps 1 - 5, seeding cells at a higher density than previously used.

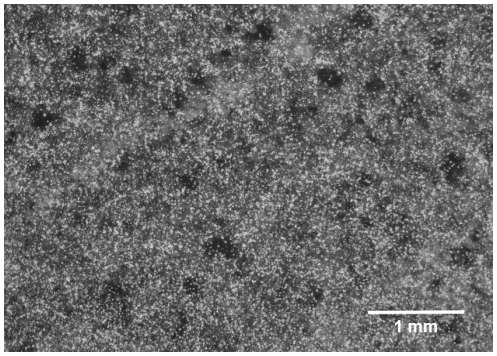


Figure 2. hPSCs at > 95% Confluency

6. Once > 95% confluency is achieved, proceed to section C for atrial cardiomyocyte differentiation and maintenance.

C. ATRIAL CARDIOMYOCYTE DIFFERENTIATION AND MAINTENANCE (DAY 0 - 15)

For preparation of STEMdiff™ Atrial Cardiomyocyte Differentiation and Maintenance media, refer to Preparation of Media section. The following instructions are for one well of a 12-well plate. For other volumes, adjust accordingly.

1. Day 0: Thaw Corning® Matrigel® on ice. Add 20 µL of Corning® Matrigel® to 2 mL of Medium A (1 in 100 dilution).
2. Gently remove medium from the wells of the 12-well plate from section B. Gently add 2 mL of Medium A supplemented with Corning® Matrigel® (prepared in step 1) per well. Incubate at 37°C for 2 days.
3. Day 2 - 14: Perform a full-medium change on Day 2 and every 2 days until Day 14, as follows:
   a. Using a pipettor, gently remove medium from the wells (do not aspirate).
   b. Gently add 2 mL of medium per well as indicated in Table 1. Incubate at 37°C.

Table 1. Full-Medium Changes with STEMdiff™ Atrial Cardiomyocyte Differentiation and Maintenance Media

DAY	MEDIUM
2	STEMdiff™ Atrial Cardiomyocyte Differentiation Medium B
4	STEMdiff™ Atrial Cardiomyocyte Differentiation Medium C
6	STEMdiff™ Atrial Cardiomyocyte Differentiation Medium C
8	STEMdiff™ Cardiomyocyte Maintenance Medium* NOTE: Small areas of beating cardiomyocytes may be visible.
10	STEMdiff™ Cardiomyocyte Maintenance Medium NOTE: Larger areas of beating cardiomyocytes should be visible over time.
12	STEMdiff™ Cardiomyocyte Maintenance Medium
14	STEMdiff™ Cardiomyocyte Maintenance Medium

*Do not feed differentiating cardiomyocytes with STEMdiff™ Cardiomyocyte Maintenance Medium before Day 8 of differentiation.

4. Day 15: hPSC-derived atrial cardiomyocytes are ready to be harvested for standard assays.
5. Day 15+: To maintain hPSC-derived atrial cardiomyocytes for 1 month or longer, perform a full-medium change every 2 days with 2 mL of STEMdiff™ Cardiomyocyte Maintenance Medium per well of a 12-well plate.