STEMdiff™ Cardiomyocyte Differentiation Kit

Culture medium kit for differentiation of human PSCs to cardiomyocytes

Catalog #05010 1 Kit

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Product Description

STEMdiff™ Cardiomyocyte Differentiation Kit includes a medium for differentiation of human embryonic stem (ES) and induced pluripotent stem (iPS) cells (human pluripotent stem cells [hPSCs]) into cardiomyocytes (cardiac troponin T-positive [cTnT+]), as well as a medium for maintenance of hPSC-derived cardiomyocytes. This kit can be used to generate 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 #05010) 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™ Cardiomyocyte Differentiation Supplement A (10X)*	05012	10 mL	Store at -20°C.	Stable for 2 years from date of manufacture (MFG) on label.
STEMdiff™ Cardiomyocyte Differentiation Supplement B (10X)*	05013	10 mL	Store at -20°C.	Stable for 2 years from date of manufacture (MFG) on label.
STEMdiff™ Cardiomyocyte Differentiation Supplement C (10X)*	05014	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
mTeSR™1 OR mTeSR™ Plus OR TeSR™-E8™	85850 OR 100-0276 OR 05990
D-PBS (Without Ca++ and Mg++)	37350
Gentle Cell Dissociation Reagent	07174
Y-27632	72302
Trypan Blue	07050

Preparation of Media

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

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

1. Thaw Differentiation Supplement A 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 Differentiation Supplement A to 90 mL of Differentiation Basal Medium. Mix thoroughly.

   NOTE: If not used immediately, store STEMdiff™ Cardiomyocyte Differentiation Medium A, B, or C at 2 - 8°C for up to 2 weeks. Warm medium to room temperature (15 - 25°C) before use.

B. PREPARATION OF STEMdiff™ CARDIOMYOCYTE MAINTENANCE MEDIUM

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

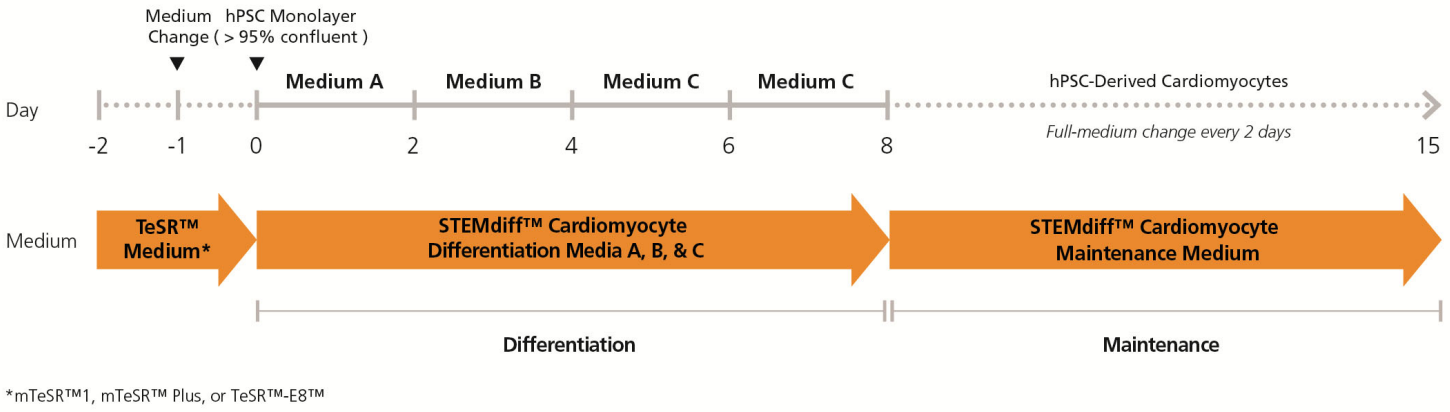
1. Thaw Maintenance Supplement at room temperature (15 - 25°C). Mix thoroughly.

   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 Maintenance Supplement to 490 mL of 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 (15 - 25°C) before use.

Protocol Diagram



*mTeSR™1, mTeSR™ Plus, or TeSR™-E8™

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 (Preparation of Reagents and Materials, section A). 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 pipette with a 1000 µL tip.
6. Immediately transfer cells to a tube containing 1 mL of mTeSR™1 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 Matrigel® from a coated 12-well plate (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 1 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.

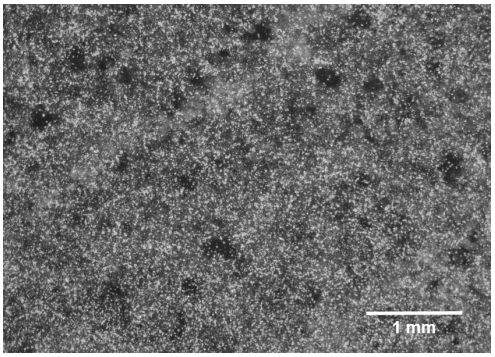


Figure 1. hPSCs at > 95% Confluency

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

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

For preparation of STEMdiff™ 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 Matrigel® on ice. Add 20 µL of Matrigel® to 2 mL of STEMdiff™ Cardiomyocyte Differentiation Medium A (1 in 100 dilution).
2. Remove medium from the wells of the 12-well plate from section B. Add 2 mL of STEMdiff™ Cardiomyocyte Differentiation Medium A supplemented with 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™ Cardiomyocyte Differentiation and Maintenance Media

DAY	MEDIUM
2	STEMdiff™ Cardiomyocyte Differentiation Medium B
4	STEMdiff™ Cardiomyocyte Differentiation Medium C
6	STEMdiff™ 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 cardiomyocytes are ready to be harvested for standard assays.
5. Day 15+: To maintain hPSC-derived 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.