

STEMdiff™ Cardiomyocyte Differentiation and Maintenance Kits



Scientists Helping Scientists™ | WWW.STEMCELL.COM

TOLL FREE PHONE 1 800 667 0322 • PHONE +1 604 877 0713

INFO@STEMCELL.COM • TECHSUPPORT@STEMCELL.COM

FOR GLOBAL CONTACT DETAILS VISIT OUR WEBSITE

Catalog #05010 1 Kit
 Catalog #05020 1 Kit

Product Description

STEMdiff™ Cardiomyocyte Differentiation Kit (Catalog #05010) 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) or TeSR™-E8™ (Catalog #05990). Greater than 80% of these cells will be cTnT+. An average of 1×10^6 cells can be harvested from a single well of a 12-well plate.

STEMdiff™ Cardiomyocyte Maintenance Kit (Catalog #05020) 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 complete kits (Catalog #05010 and 05020) and are not available for individual sale.

COMPONENT NAME	COMPONENT #	SIZE	STORAGE	SHELF LIFE
STEMdiff™ Cardiomyocyte Differentiation Kit (05010)				
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.
STEMdiff™ Cardiomyocyte Maintenance Kit (05020)				
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.

Materials Required But Not Included

PRODUCT NAME	CATALOG #
Corning® Matrigel® hESC-Qualified Matrix	Corning 354277
mTeSR™1 OR TeSR™-E8™	85850 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 techniques to prepare complete 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 COMPLETE STEMdiff™ CARDIOMYOCYTE MAINTENANCE MEDIUM

Use sterile techniques to prepare complete 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.

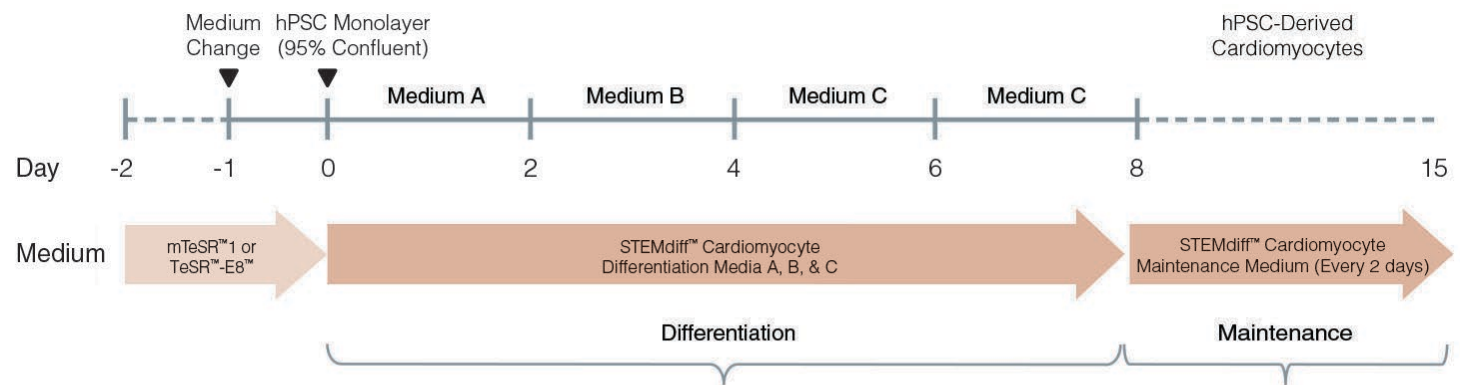
1. Thaw Maintenance Supplement at room temperature (15 - 25°C). Mix thoroughly.

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 complete STEMdiff™ Cardiomyocyte Maintenance Medium at 2 - 8°C for up to 4 weeks. Warm complete medium to room temperature (15 - 25°C) before use.

Protocol Diagram



Directions for Use

Please read the entire protocol before proceeding. Use sterile techniques 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 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 how to maintain hPSCs in mTeSR™1 or TeSR™-E8™, and for coating plates with Corning® Matrigel®, refer to the Technical Manual: Maintenance of Human Pluripotent Stem Cells in mTeSR™1 (Document #28315) or TeSR™-E8™ (Document #DX20809), available at www.stemcell.com or contact us to request a copy.

1. Coat a 12-well tissue culture plate with Corning® Matrigel® hESC-Qualified Matrix 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% CO₂ 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 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 pre-coated 12-well plate (section A, step 1). Add 1 mL of mTeSR™1 or TeSR™-E8™ supplemented with 10 µM Y-27632 per well.
2. Add cells (from section A) at a density of 3.5 x 10⁵ cells/well.
NOTE: Move the plate in several quick, short, back-and-forth and side-to-side motions to ensure uniform distribution of cells.
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 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 (48 hours after seeding). If cells are < 95% confluent, do not continue incubation. Repeat steps 1 - 5, seeding cells at a higher density than previously used.
6. Once > 95% confluency is achieved, proceed to section C for cardiomyocyte differentiation and maintenance.

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

For preparation of STEMdiff™ Cardiomyocyte Differentiation and Maintenance media, refer to Preparation of Media section. The following instructions are for 1 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 STEMdiff™ Cardiomyocyte Differentiation Medium A (1 in 100 dilution).
2. Remove medium from the wells of the 12-well plate from section B.
3. Add 2 mL of STEMdiff™ Cardiomyocyte Differentiation Medium A supplemented with Corning® Matrigel® (prepared in step 1) per well. Incubate at 37°C for 2 days.
4. **Day 2:** Remove medium and gently add 2 mL of STEMdiff™ Cardiomyocyte Differentiation Medium B. Incubate at 37°C for 2 days.
5. **Day 4:** Remove medium and gently add 2 mL of STEMdiff™ Cardiomyocyte Differentiation Medium C. Incubate at 37°C for 2 days.
6. **Day 6:** Remove medium and gently add 2 mL of STEMdiff™ Cardiomyocyte Differentiation Medium C. Incubate at 37°C for 2 days.
7. **Day 8:** Remove medium and gently add 2 mL of complete STEMdiff™ Cardiomyocyte Maintenance Medium. Incubate at 37°C for 2 days.
NOTE: Small areas of beating cardiomyocytes may be visible.
NOTE: Do not feed differentiating cardiomyocytes with STEMdiff™ Cardiomyocyte Maintenance Medium before Day 8 of differentiation.
8. **Day 10, 12, and 14:** Remove medium and gently add 2 mL of complete STEMdiff™ Cardiomyocyte Maintenance Medium. Incubate at 37°C.
NOTE: Larger areas of beating cardiomyocytes should be visible over time.
9. **Day 15:** hPSC-derived cardiomyocytes are ready to be harvested for standard assays.
10. **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.

STEMCELL TECHNOLOGIES INC.'S QUALITY MANAGEMENT SYSTEM IS CERTIFIED TO ISO 13485. PRODUCTS ARE FOR RESEARCH USE ONLY AND NOT INTENDED FOR HUMAN OR ANIMAL DIAGNOSTIC OR THERAPEUTIC USES UNLESS OTHERWISE STATED.

Copyright © 2019 by STEMCELL Technologies Inc. All rights reserved including graphics and images. STEMCELL Technologies & Design, STEMCELL Shield Design, Scientists Helping Scientists, and STEMdiff are trademarks of STEMCELL Technologies Canada Inc. mTeSR, TeSR, and E8 are trademarks of WARF. Corning and Matrigel are registered trademarks of Corning Incorporated. All other trademarks are the property of their respective holders. While STEMCELL has made all reasonable efforts to ensure that the information provided by STEMCELL and its suppliers is correct, it makes no warranties or representations as to the accuracy or completeness of such information.