

STEMdiff™ Cardiomyocyte Plating Kit

Medium for thawing and replating human PSC-derived cardiomyocytes

Catalog #100-1121

1 Kit



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Product Description

STEMdiff™ Cardiomyocyte Plating Medium is a versatile medium that improves the plating efficiency of human pluripotent stem cell-derived cardiomyocytes (hPSC-CMs) when transitioning from cryopreservation to thawing, and from harvesting to replating of hPSC-CMs. After thawing or replating, the functional capacity of hPSC-CMs is retained and can be used in various downstream applications and analyses. hPSC-CMs can be further maintained long-term using STEMdiff™ Cardiomyocyte Maintenance Kit (Catalog #05020).

Product Information

The following components are sold as a complete kit (Catalog #100-1121). STEMdiff™ Cardiomyocyte Support Medium (Catalog #05027) is also available for individual sale.

COMPONENT NAME	COMPONENT #	SIZE	STORAGE	SHELF LIFE
STEMdiff™ Cardiomyocyte Support Medium	05027	250 mL	Store at -20°C.	Stable for 1 year from date of manufacture (MFG) on label.
STEMdiff™ Cardiomyocyte Plating Supplement (100X)*	100-1120	2.5 mL	Store at -20°C.	Stable for 1 year 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
STEMdiff™ Cardiomyocyte Maintenance Kit	05020
STEMdiff™ Cardiomyocyte Dissociation Kit	05025
D-PBS (Without Ca++ and Mg++)	37350
Serological pipettes, 2 mL and 10 mL	e.g. 38002 and 38004
Conical tubes, 15 mL	e.g. 38009
Tissue culture-treated plates, 12-well	e.g. 38052
Trypan Blue	07050
Hausser Scientific™ Bright-Line Hemocytometer	100-1181

Preparation of STEMdiff™ Cardiomyocyte Plating Medium

Use sterile technique to prepare complete STEMdiff™ Cardiomyocyte Plating Medium (STEMdiff™ Cardiomyocyte Support Medium + STEMdiff™ Cardiomyocyte Plating Supplement [100X]). The following example is for preparing 250 mL of complete medium. If preparing other volumes, adjust accordingly.

1. Thaw STEMdiff™ Cardiomyocyte Support Medium at room temperature (15 - 25°C) or overnight at 2 - 8°C. Mix thoroughly.
2. Thaw STEMdiff™ Cardiomyocyte Plating Supplement (100X) at room temperature (15 - 25°C). Mix thoroughly.
NOTE: Once thawed, use immediately or aliquot and store at -20°C. Do not exceed the shelf life of the Supplement. After thawing aliquots, use immediately. Do not re-freeze.
3. Add 2.5 mL of STEMdiff™ Cardiomyocyte Plating Supplement (100X) to 247.5 mL of STEMdiff™ Cardiomyocyte Support Medium. Mix thoroughly.
NOTE: If not used immediately, store complete STEMdiff™ Cardiomyocyte Plating Medium at 2 - 8°C for up to 2 weeks. Warm medium to room temperature before use.

Directions for Use

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

- A. Thawing Cryopreserved hPSC-CMs
- B. Dissociating hPSC-CMs
- C. Replating hPSC-CMs

A. THAWING CRYOPRESERVED hPSC-CMs

The following instructions are for thawing one cryovial of frozen hPSC-CMs and plating onto a 12-well plate. If thawing additional cryovials, adjust volumes accordingly. Frozen hPSC-CMs should be thawed and plated onto Corning® Matrigel®-coated cultureware.

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.
NOTE: For complete instructions on coating plates with Corning® Matrigel®, refer to the Technical Manual for mTeSR™1, mTeSR™ Plus, TeSR™-E8™, or TeSR™-AOF, available at www.stemcell.com, or contact us to request a copy.
2. Thaw hPSC-CMs in a 37°C water bath by gently swirling the cryovial continuously until only a small frozen cell pellet remains.
3. Add 5 - 7 mL of STEMdiff™ Cardiomyocyte Plating Medium (see Preparation of Medium) to a 15 mL conical tube.
4. Using a 2 mL pipette, gently transfer the contents of the cryovial to the tube from step 3.
5. Centrifuge the cells at 300 x g for 5 minutes at room temperature.
6. Aspirate the supernatant and gently add 1 - 2 mL of STEMdiff™ Cardiomyocyte Plating Medium to resuspend cells.
7. Perform a cell count using an automated cell counter (e.g. NucleoCounter® NC-250™) or with Trypan Blue and a Hausser Scientific™ Bright-Line Hemocytometer.
8. Thawed hPSC-CMs are now ready for replating (proceed to section C) or standard assays.

B. DISSOCIATING hPSC-CMs

The following instructions are for the dissociation of hPSC-CMs that have been maintained in STEMdiff™ Cardiomyocyte Maintenance Medium in one well of a 12-well plate. Dissociation can be performed as early as Day 15 of differentiation/maintenance.

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.
NOTE: For complete instructions on coating plates with Corning® Matrigel®, refer to the Technical Manual for mTeSR™1, mTeSR™ Plus, TeSR™-E8™, or TeSR™-AOF, available at www.stemcell.com, or contact us to request a copy.
2. Warm thawed STEMdiff™ Cardiomyocyte Dissociation Medium to 37°C.
NOTE: For complete instructions on preparing STEMdiff™ Cardiomyocyte Dissociation Medium, refer to the Product Information Sheet (PIS) for STEMdiff™ Cardiomyocyte Dissociation Kit (Document #10000003446), available at www.stemcell.com, or contact us to request a copy.
3. Wash each well to be harvested two times with 1 mL of D-PBS (Without Ca++ and Mg++).
4. Gently remove the wash and add 1 mL/well of warm (37°C) STEMdiff™ Cardiomyocyte Dissociation Medium.
5. Incubate at 37°C and 5% CO₂ for 10 - 12 minutes.
6. Add 2 mL of STEMdiff™ Cardiomyocyte Plating Medium per well. Dislodge cells by pipetting up and down 3 - 5 times using a 10 mL serological pipette.

CRITICAL: Do not use a smaller-bore pipette tip at this step, as this may result in significant cell death.

7. Immediately transfer the cells from one well to a tube containing 3 mL of STEMdiff™ Cardiomyocyte Plating Medium.
8. Centrifuge at 300 x g for 5 minutes. Remove and discard supernatant.
9. Gently resuspend the cell pellet with 1 - 2 mL of STEMdiff™ Cardiomyocyte Plating Medium.
10. Perform a cell count using an automated cell counter (e.g. NucleoCounter® NC-250™) or with Trypan Blue and a Hausser Scientific™ Bright-Line Hemocytometer.
11. Single-cell hPSC-CMs are now ready for replating (proceed to section C) or standard assays.

C. REPLATING hPSC-CMs

The following instructions are for replating thawed (section A) or dissociated (section B) hPSC-CMs onto a 12-well plate. For other cultureware, adjust volumes accordingly.

1. Warm STEMdiff™ Cardiomyocyte Plating Medium (see Preparation of Media section A) to room temperature.
2. Aspirate Corning® Matrigel® from the pre-coated 12-well tissue culture plate (prepared in section A or B, step 1), and add 0.5 mL of STEMdiff™ Cardiomyocyte Plating Medium per well.
3. Add cells at a density appropriate for downstream assays (e.g. electrophysiology, flow cytometry, or immunocytochemistry) or other applications.
4. Incubate at 37°C and 5% CO₂ for 24 hours.
5. The following day, warm STEMdiff™ Cardiomyocyte Plating Medium to room temperature.

NOTE: For storage, stability, and preparation instructions for STEMdiff™ Cardiomyocyte Maintenance Medium, refer to the PIS (Document #10000009775), available at www.stemcell.com or contact us to request a copy.

6. Remove medium and add 2 mL of STEMdiff™ Cardiomyocyte Maintenance Medium per well. Incubate at 37°C and 5% CO₂.
7. Every 2 days, perform a full-medium change with 2 mL of STEMdiff™ Cardiomyocyte Maintenance Medium per well.

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