STEMdiff™ Cardiomyocyte Expansion Kit

Serum-free kit for the expansion of early-stage human PSC-derived cardiomyocytes

Catalog #100-1109 1 Kit

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Product Description

STEMdiff™ Cardiomyocyte Expansion Kit is designed for the serum-free expansion of early-stage human pluripotent stem cell-derived cardiomyocytes (hPSC-CMs). These cells, which are characterized by the cardiac muscle cell marker cardiac troponin T (cTnT), can be expanded for up to 5 passages using STEMdiff™ Cardiomyocyte Expansion Medium. This kit also contains STEMdiff™ Cardiomyocyte Passaging Supplement (100X), which can be combined with STEMdiff™ Cardiomyocyte Support Medium (Catalog #05027) to harvest and replate the expanding hPSC-CMs. This kit is compatible with hPSC-CMs differentiated using STEMdiff™ Ventricular Cardiomyocyte Differentiation Kit (Catalog #05010) and STEMdiff™ Atrial Cardiomyocyte Differentiation Kit (Catalog #100-0215).

Product Information

The following components are sold as a complete kit (Catalog #100-1109) and are not available for individual sale.

COMPONENT NAME	COMPONENT #	SIZE	STORAGE	SHELF LIFE
STEMdiff™ Cardiomyocyte Maintenance Basal Medium*	05015	490 mL	Store at 2 - 8°C.	Stable for 2 years from date of manufacture (MFG) on label.
STEMdiff™ Cardiomyocyte Passaging Supplement (100X)†	100-1107	2.5 mL	Store at -20°C.	Stable for 1 year from date of manufacture (MFG) on label.
STEMdiff™ Cardiomyocyte Expansion Supplement (50X)†	100-1108	10 mL	Store at -20°C.	Stable for 2 years from date of manufacture (MFG) on label.

* Also available as part of STEMdiff™ Cardiomyocyte Maintenance Kit (Catalog #05020).
† 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 #
Conical tubes, 15 mL or 50 mL	e.g. 38009 or 38010
Corning® Matrigel® hESC-Qualified Matrix	Corning 354277
D-PBS (Without Ca++ and Mg++)	37350
Serological pipettes, 5 mL and 10 mL	e.g. 38003 and 38004
STEMdiff™ Cardiomyocyte Dissociation Kit	05025
STEMdiff™ Cardiomyocyte Support Medium*	05027
Tissue culture-treated plates, 12-well and 6-well	e.g. 38052 and 38016
Trypan Blue	07050
Hausser Scientific™ Bright-Line Hemocytometer	100-1181

* NOTE: STEMdiff™ Cardiomyocyte Support Medium contains serum.

Preparation of Media

A. PREPARATION OF STEMdiff™ CARDIOMYOCYTE PASSAGING MEDIUM

Use sterile technique to prepare complete STEMdiff™ Cardiomyocyte Passaging Medium (STEMdiff™ Cardiomyocyte Support Medium + STEMdiff™ Cardiomyocyte Passaging 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 Passaging Supplement (100X) at room temperature. 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 Passaging Supplement (100X) to 247.5 mL of STEMdiff™ Cardiomyocyte Support Medium. Mix thoroughly.

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

B. PREPARATION OF STEMdiff™ CARDIOMYOCYTE EXPANSION MEDIUM

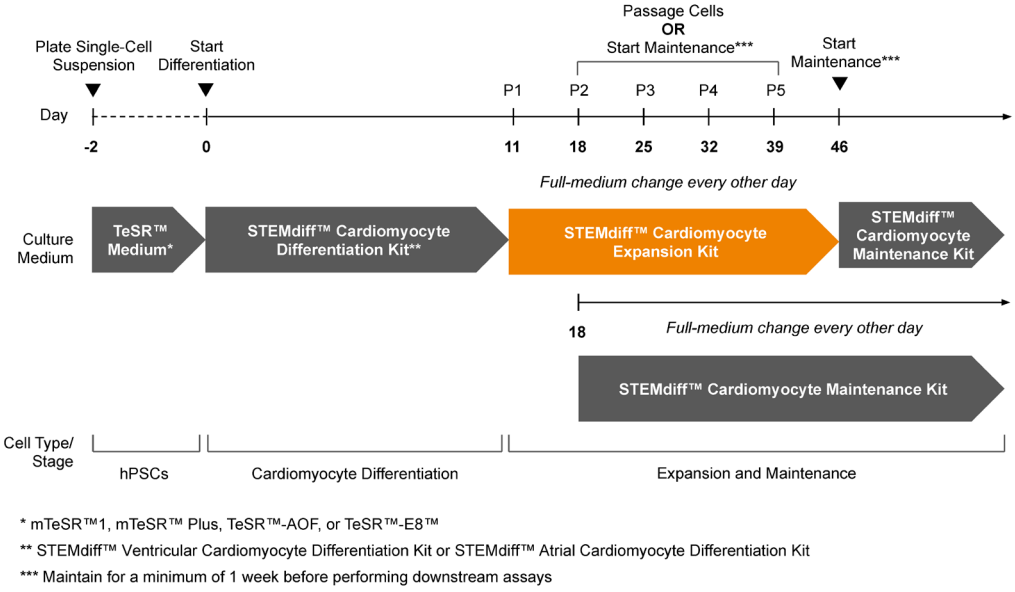
Use sterile technique to prepare complete STEMdiff™ Cardiomyocyte Expansion Medium (STEMdiff™ Cardiomyocyte Maintenance Basal Medium + STEMdiff™ Cardiomyocyte Expansion Supplement [50X]). The following example is for preparing 500 mL of complete medium. If preparing other volumes, adjust accordingly.

1. Thaw STEMdiff™ Cardiomyocyte Expansion Supplement (50X) 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 the aliquots, use immediately. Do not re-freeze.
2. Add 10 mL of STEMdiff™ Cardiomyocyte Expansion Supplement (50X) to 490 mL of STEMdiff™ Cardiomyocyte Maintenance Basal Medium. Mix thoroughly.

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

Protocol Diagram



* mTeSR™1, mTeSR™ Plus, TeSR™-AOF, or TeSR™-E8™

** STEMdiff™ Ventricular Cardiomyocyte Differentiation Kit or STEMdiff™ Atrial Cardiomyocyte Differentiation Kit

*** Maintain for a minimum of 1 week before performing downstream assays

Figure 1. Cardiomyocyte Generation, Expansion, and Maintenance

Expansion of hPSC-CMs is initiated on day 11 of cardiomyocyte differentiation. Day 11 ventricular or atrial hPSC-CMs generated using STEMdiff™ Ventricular Cardiomyocyte Differentiation Kit (Catalog #05010) or STEMdiff™ Atrial Cardiomyocyte Differentiation Kit (Catalog #100-0215), respectively, are dissociated with STEMdiff™ Cardiomyocyte Dissociation Kit (Catalog #05025). The dissociated hPSC-CMs are then plated and expanded for up to 5 passages using STEMdiff™ Cardiomyocyte Expansion Kit (see Figure 2). STEMdiff™ Cardiomyocyte Maintenance Kit (Catalog #05020) can be used to stop expansion for long-term maintenance or downstream assays as early as Day 18 (P1 end) or as late as Day 46 (P5 end).

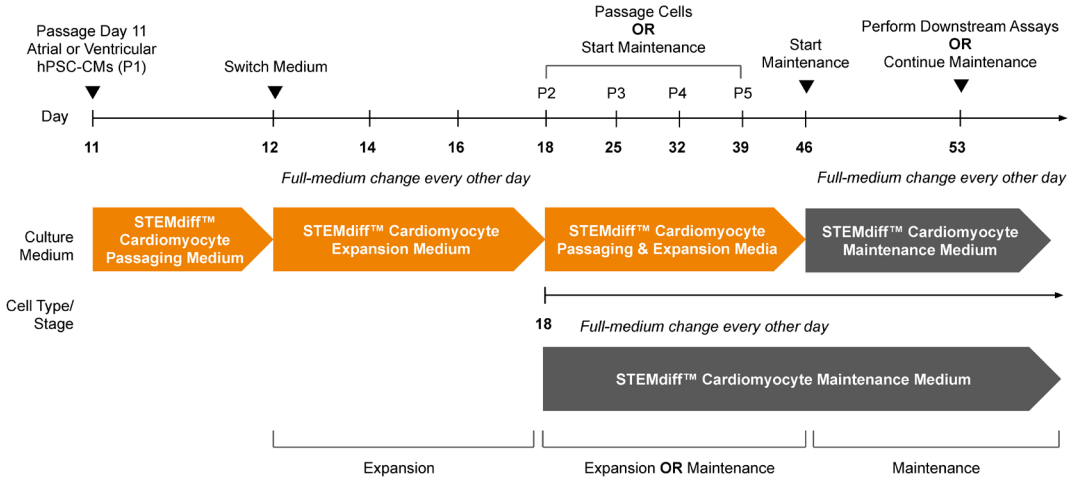


Figure 2. STEMdiff™ Cardiomyocyte Expansion Kit Workflow

Day 11 ventricular or atrial hPSC-CMs are passaged (P1) and plated in STEMdiff™ Cardiomyocyte Passaging Medium for 24 hours. The medium is switched to STEMdiff™ Cardiomyocyte Expansion Medium on Day 12 to allow the hPSC-CMs to expand, and a full-medium change is performed every other day until Day 18. On Day 18, P1 hPSC-CMs may be passaged again (P2) to continue expansion, or expansion may be stopped by switching the medium to STEMdiff™ Cardiomyocyte Maintenance Medium for long-term maintenance or downstream assays. hPSC-CMs can be passaged up to P5 before initiating maintenance, and must be maintained for a minimum of 1 week in STEMdiff™ Cardiomyocyte Maintenance Medium before starting downstream assays.

Directions for Use

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

A. Dissociation and Plating of Early-Stage hPSC-CMs

B. Expansion of hPSC-CMs (Day 12+)

C. Passaging Expanding hPSC-CMs (Day 18+)

D. Stopping Expansion of hPSC-CMs

For complete instructions on generating ventricular or atrial hPSC-CMs, refer to the Product Information Sheet (PIS) for STEMdiff™ Ventricular Cardiomyocyte Differentiation Kit (Document #10000003442) or STEMdiff™ Atrial Cardiomyocyte Differentiation Kit (Document #10000008184), available at www.stemcell.com, or contact us to request a copy.

A. DISSOCIATION AND PLATING OF EARLY-STAGE hPSC-CMs

The following instructions are for dissociating one well of a 12-well plate of early-stage (Day 11) ventricular or atrial hPSC-CMs generated using STEMdiff™ Ventricular or Atrial Cardiomyocyte Differentiation Kits (see Figure 1) and plating into one well of a 6-well plate. For other cultureware, adjust volumes according to the Tables referenced below. Early-stage hPSC-CMs should exhibit a confluent, beating monolayer before proceeding with expansion (see Figure 3).

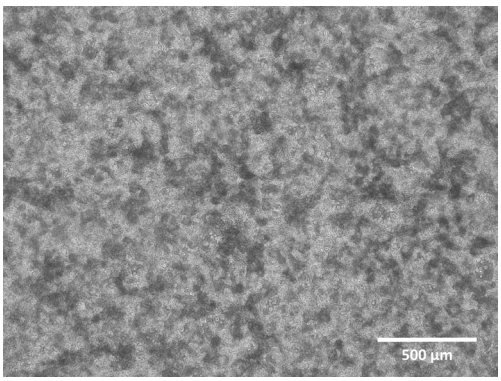


Figure 3. Representative Image of Day 11 hPSC-derived CMs

1. Day 11: Coat the desired culture vessel for expansion (e.g. a 6-well tissue culture-treated 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 STEMdiff™ Cardiomyocyte Passaging Medium (see Preparation of Media section A) to room temperature (15 - 25°C). Warm thawed STEMdiff™ Cardiomyocyte Dissociation Medium to 37°C.

   NOTE: For complete instructions on preparing STEMdiff™ Cardiomyocyte Dissociation Medium, refer to the PIS for STEMdiff™ Cardiomyocyte Dissociation Kit (Document #10000003446), available at www.stemcell.com, or contact us to request a copy.

3. Harvest the Day 11 hPSC-CMs as follows:
   a. Wash the well with 1 mL of D-PBS (Without Ca++ and Mg++).
   b. Gently remove the wash and add 1 mL/well of warm (37°C) STEMdiff™ Cardiomyocyte Dissociation Medium.
   c. Incubate at 37°C and 5% CO_2 for 10 - 12 minutes.
   d. Add 2 mL of STEMdiff™ Cardiomyocyte Passaging 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 serological pipette or micropipette to dislodge the cells, as this may result in significant cell death.
   e. Immediately transfer cells from one well to a 15 mL conical tube containing 3 mL of STEMdiff™ Cardiomyocyte Passaging Medium.
   f. Centrifuge at 300 x *g* for 5 minutes. Remove and discard supernatant.
   g. Gently resuspend the cell pellet with 1 mL of STEMdiff™ Cardiomyocyte Passaging Medium.

4. 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.

   CRITICAL: Before proceeding with expansion, it is recommended to perform flow cytometry on the harvested hPSC-CMs to confirm that cTnT expression has reached ≥ 80%. Proceeding with hPSC-CMs that are < 80% cTnT+ may result in decreased expansion and purity.

5. Aspirate Corning® Matrigel® from the coated culture vessel (prepared in step 1). Add 1 mL of STEMdiff™ Cardiomyocyte Passaging Medium per well of a 6-well plate. For other cultureware, refer to Table 1 for recommended volumes.

Table 1. Recommended Volumes of Passaging Medium for Various Cultureware

CULTUREWARE	VOLUME OF STEMdiff™ CARDIOMYOCYTE PASSAGING MEDIUM
6-well plate	1 mL/well
T-75 cm^2 flask	8 mL/well
T-175 cm^2 flask	18 mL/well
T-875 cm^2 flask (e.g. Falcon Catalog 353144)	90 mL/well

6. Plate cells at a density of 5 x 10^5 cells/well of a 6-well plate (5 x 10^4 cells/cm^2). Refer to Table 2 for recommended plating densities for other cultureware.

   NOTE: Adjust the volume of STEMdiff™ Cardiomyocyte Passaging Medium added to the culture vessel relative to the cell suspension volume, such that the total volume added does not exceed the recommended volume listed in Table 1.

   For example, to plate a cell solution containing 5 x 10^5 cells in 200 µL, first add 800 µL of STEMdiff™ Cardiomyocyte Passaging Medium to one well of a 6-well plate, then add 200 µL cell solution for a final plating volume of 1000 µL (1 mL).

Table 2. Recommended Cell Plating Density of hPSC-CMs for Various Cultureware

CULTUREWARE	PLATING DENSITY OF hPSC-CMs
6-well plate	5.0 x 10^5 cells/well
T-75 cm^2 flask	3.9 x 10^6 cells/flask
T-175 cm^2 flask	9.1 x 10^6 cells/flask
T-875 cm^2 flask	4.6 x 10^7 cells/flask

7. Incubate at 37°C and 5% CO_2 for 24 hours. Do not disturb the cells. Proceed to section B.

B. EXPANSION OF hPSC-CMs (DAY 12+)

For preparation of STEMdiff™ Cardiomyocyte Expansion, refer to Preparation of Media section B. The following instructions are for expanding Day 12 hPSC-CMs (P1) in one well of a 6-well plate. For other cultureware, adjust volumes according to Table 3. These instructions also apply to later passages (P2 to P5).

1. Day 12: Warm STEMdiff™ Cardiomyocyte Expansion Medium (see Preparation of Media section B) to room temperature (15 - 25°C).
2. Remove medium from the well and add 2 mL of complete STEMdiff™ Cardiomyocyte Expansion Medium.

Table 3. Recommended Volumes of Expansion Medium for Various Cultureware

CULTUREWARE	VOLUME OF STEMdiff™ CARDIOMYOCYTE EXPANSION MEDIUM
6-well plate	2 mL/well
T-75 cm^2 flask	15 mL/flask
T-175 cm^2 flask	36 mL/flask
T-875 cm^2 flask	180 mL/flask

3. Incubate at 37°C and 5% CO_2 for 48 hours.
4. Day 14 and 16: Perform a full-medium change by repeating steps 1 - 3.
5. Day 18: Expanding hPSC-CMs are ready to harvest or maintain.
   - To continue expanding hPSC-CMs from P2 up to P5, proceed to section C.
   - To stop expansion for downstream assays or long-term hPSC-CM maintenance, proceed to section D.

C. PASSAGING EXPANDING hPSC-CMs (DAY 18+)

The following instructions are for the passaging of expanding atrial or ventricular hPSC-CMs in one well of a 6-well plate. For other cultureware, refer to Table 4 for recommended volumes. By Day 18, hPSC-CM cultures should typically reach 80 - 100% confluency before passaging. These instructions also apply to later passages (P2 to P5).

1. Day 18: Coat the 6-well tissue culture-treated plate with Corning® Matrigel® hESC-Qualified Matrix and bring to room temperature (15 - 25°C) for at least 1 hour prior to use.
2. Warm STEMdiff™ Cardiomyocyte Passaging Medium (see Preparation of Media section A) to room temperature. Warm thawed STEMdiff™ Cardiomyocyte Dissociation Medium to 37°C.

   NOTE: For complete instructions on preparing STEMdiff™ Cardiomyocyte Dissociation Medium, refer to the PIS for STEMdiff™ Cardiomyocyte Dissociation Kit (Document #10000003446), available at www.stemcell.com, or contact us to request a copy
3. Harvest the expanding hPSC-CMs as follows:
   a. Wash the well with 1 mL of D-PBS (Without Ca++ and Mg++).
   b. Gently remove the wash and add 1 mL of warm (37°C) STEMdiff™ Cardiomyocyte Dissociation Medium to the well. Refer to Table 4 for recommended volumes.
   c. Incubate at 37°C and 5% CO_2 for 3 - 10 minutes.

      NOTE: For later passages, a longer incubation time may be required.
   d. Add 2 mL of STEMdiff™ Cardiomyocyte Passaging Medium. Dislodge cells by pipetting up and down 3 - 5 times using a 5 or 10 mL serological pipette, or tap the cultureware to detach cells.

      CRITICAL: Do not use a smaller-bore serological pipette or micropipette to dislodge the cells, as this may result in significant cell death.
   e. Immediately transfer cells to a 15 mL conical tube containing 3 mL of Passaging Medium. Refer to Table 4 for recommended volumes.

Table 4. Recommended Volumes of Media for Harvesting Expanding hPSC-CMs in Various Cultureware

CULTUREWARE	VOLUME OF STEMdiff™ CARDIOMYOCYTE DISSOCIATION MEDIUM	VOLUME OF STEMdiff™ CARDIOMYOCYTE PASSAGING MEDIUM*
6-well plate	1 mL/well	5 mL/well
T-75 cm^2 flask	8 mL/flask	40 mL/flask
T-175 cm^2 flask	18 mL/flask	18 mL/flask
T-875 cm^2 flask	90 mL/flask	450 mL/flask

* Total volume required to perform steps 3d and 3e.

f. Centrifuge at 300 x *g* for 5 minutes. Remove and discard supernatant.

g. Gently resuspend the cell pellet with 1 mL of STEMdiff™ Cardiomyocyte Passaging Medium.

h. 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.

i. Remove Corning® Matrigel® from the coated vessel(s) prepared in step 1. Add 1 mL of STEMdiff™ Cardiomyocyte Passaging Medium per well of a 6-well plate. Refer to Table 1 for recommended volumes for various cultureware.

j. Plate cells at a density of 5 x 10^5 cells/well (5 x 10^4 cells/cm^2). Refer to Table 2 for recommended plating densities for various cultureware.

NOTE: Adjust the volume of STEMdiff™ Cardiomyocyte Passaging Medium added to the culture vessel relative to the cell suspension volume, such that the total volume added does not exceed the recommended volumes listed in Table 1.

For example, to plate a cell solution containing 5 x 10^5 cells in 200 µL, first add 800 µL of STEMdiff™ Cardiomyocyte Passaging Medium to one well of a 6-well plate, then add 200 µL cell solution for a final plating volume of 1000 µL (i.e. 1 mL).

4. Incubate at 37°C and 5% CO_2 for 24 hours. Do not disturb the cells.

NOTE: hPSC-CMs may continue to be expanded and passaged up to 5 times by repeating sections B and C, respectively, from Day 18 - 46 (see Figure). Expansion may be stopped with STEMdiff™ Cardiomyocyte Maintenance Medium for long-term maintenance or downstream assays from Day 18 - 46 (see section D).

D. STOPPING EXPANSION OF hPSC-CMs

The following instructions are for stopping the expansion of atrial or ventricular hPSC-CMs in a 6-well plate using STEMdiff™ Cardiomyocyte Maintenance Medium on day 18 (P1 end), but are also applicable to later passages. For other cultureware, refer to Table 5 for recommended volumes. Expansion may be stopped for downstream assays or to initiate long-term hPSC-CMs maintenance from Day 18 - 46 (see Figure 2).

1. Day 18: Prepare STEMdiff™ Cardiomyocyte Maintenance Medium and warm to room temperature (15 - 25°C).

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

2. Remove medium and add 4 mL of STEMdiff™ Cardiomyocyte Maintenance Medium per well of a 6-well plate. Refer to Table 5 for recommended volumes.

Table 5. Recommended Volumes of Medium for Maintenance of hPSC-CMs after Expansion in Various Cultureware

CULTUREWARE	VOLUME OF STEMdiff™ CARDIOMYOCYTE EXPANSION MEDIUM
6-well plate	4 mL/well
T-75 cm^2 flask	30 mL/flask
T-175 cm^2 flask	72 mL/flask
T-875 cm^2 flask	360 mL/flask

3. Incubate at 37°C and 5% CO_2 for 48 hours.
4. Day 20 - 24: Perform a full-medium change with STEMdiff™ Cardiomyocyte Maintenance Medium every 2 days.
5. Day 25: After 1 week in STEMdiff™ Cardiomyocyte Maintenance Medium, atrial or ventricular hPSC-CMs are ready to be harvested for downstream assays such as electrophysiology, flow cytometry, or immunocytochemistry.
6. Day 25+: To maintain atrial or ventricular hPSC-CMs for 1 month or longer, perform a full-medium change every 2 days with STEMdiff™ Cardiomyocyte Maintenance Medium.