STEMdiff™ Myogenic Progenitor Supplement Kit

Serum-free supplements for differentiation of human pluripotent stem cells (hPSCs) to myogenic progenitor cells

Catalog #100-0151 1 Kit

STEMCELL™ TECHNOLOGIES
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

Product Description

STEMdiff™ Myogenic Progenitor Supplement Kit comprises serum-free supplements for the differentiation of human embryonic stem (ES) cells and induced pluripotent stem (iPS) cells to myogenic progenitor cells. These cells, which are characterized by myogenic cell markers such as CD56 and CD82, can be culture-expanded for 5+ passages using MyoCult™-SF Expansion Supplement Kit (Human; Catalog #05980) and further differentiated to functional multinucleated MyHC+ myotubes at high efficiency using MyoCult™ Differentiation Kit (Human; Catalog #05965). These myotubes can be used in various downstream applications and analyses. This kit is compatible with human ES and iPS cells maintained in mTeSR™1 (Catalog #85850), mTeSR™ Plus (Catalog #100-0276), or TeSR™-E8™ (Catalog #05990).

Product Information

The following components are sold as a complete kit (Catalog #100-0151) and are not available for individual sale.

COMPONENT NAME	COMPONENT #	SIZE	STORAGE	SHELF LIFE
STEMdiff™ Myogenic Progenitor Supplement A (100X)	#100-0152	240 µL	Store at -20°C.	Stable for 2 years from date of manufacture (MFG) on label.
STEMdiff™ Myogenic Progenitor Supplement B (100X)	#100-0153	240 µL	Store at -20°C.	Stable for 2 years from date of manufacture (MFG) on label.
STEMdiff™ Myogenic Progenitor Supplement C (100X)	#100-0154	240 µL	Store at -20°C.	Stable for 2 years from date of manufacture (MFG) on label.
STEMdiff™ Myogenic Progenitor Supplement D (5X)	#100-0155	60 mL	Store at -20°C.	Stable for 2 years from date of manufacture (MFG) on label.

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
DMEM/F-12 (without HEPES)	Thermo Fisher 11320-033
Y-27632 (Dihydrochloride)	72304
D-PBS (Without Ca++ and Mg++)	37350
TrypLE™ Express	Thermo Fisher 12605010
Collagenase Type IV (1 mg/mL)	07909
70 µm Reversible Strainer, Large	27260
Falcon® Conical Tubes, 15 mL and 50 mL	38009 (15 mL) and 38010 (50 mL)
Tissue culture-treated plates	e.g. 38015
MyoCult™-SF Expansion Supplement Kit (Human)	05980
MyoCult™ Differentiation Kit (Human)	05965
Trypan Blue	07050
Gentle Cell Dissociation Reagent	100-0485
Animal Component-Free Cell Dissociation Kit	05426

Preparation of Reagents and Materials

A. PREPARATION OF STEMdiff™ MYOGENIC PROGENITOR MEDIA A, B, & C

Use sterile technique to prepare STEMdiff™ Myogenic Progenitor Medium A, B, or C (Progenitor Supplement A, B, or C + DMEM/F-12 [without HEPES]). The following example is for preparing 10 mL of STEMdiff™ Myogenic Progenitor Medium A. If preparing other volumes, adjust accordingly. For Medium B and Medium C, follow the instructions below, replacing Progenitor Supplement A with Progenitor Supplement B or Progenitor Supplement C, respectively.

1. Thaw STEMdiff™ Myogenic Progenitor Supplement A at room temperature (15 - 25°C). Mix thoroughly.

   NOTE: If not used immediately, store at 2 - 8°C for up to 4 weeks. Alternatively, aliquot and store at -20°C. Do not exceed the shelf life of the components. After thawing aliquots, use immediately. Do not re-freeze.
2. Add 100 µL of STEMdiff™ Myogenic Progenitor Supplement A to 9.90 mL DMEM/F-12 (without HEPES). Mix thoroughly. Warm to room temperature before use.

   NOTE: If not used immediately, store at 2 - 8°C for up to 4 weeks.

B. PREPARATION OF STEMdiff™ MYOGENIC PROGENITOR MEDIUM D

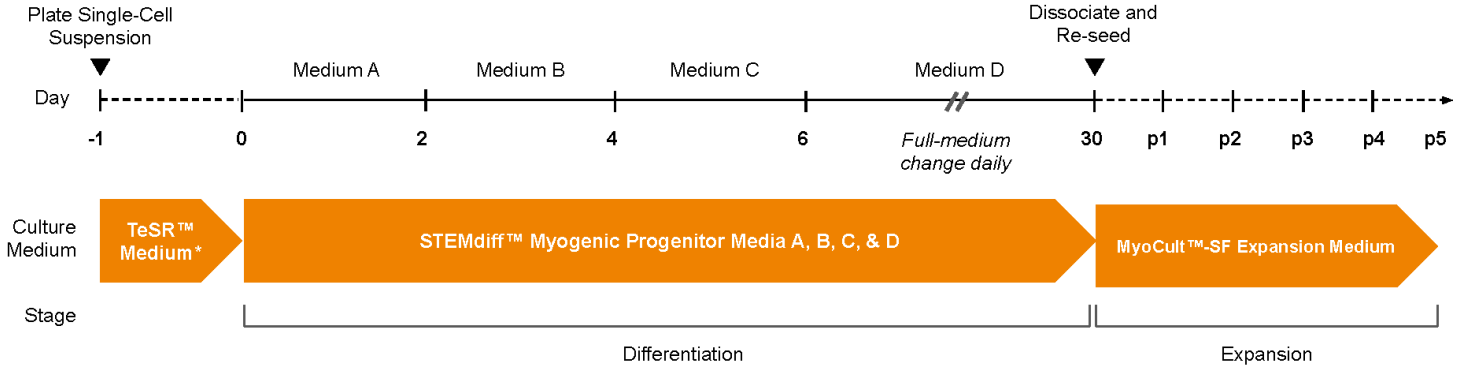
Use sterile technique to prepare STEMdiff™ Myogenic Progenitor Medium D (Progenitor Supplement D + DMEM/F-12 [without HEPES]). The following example is for preparing 100 mL of complete medium. If preparing other volumes, adjust accordingly.

1. Thaw STEMdiff™ Myogenic Progenitor Supplement D at room temperature (15 - 25°C) or overnight at 4°C. Mix thoroughly.

   NOTE: If not used immediately, aliquot and store at -20°C. Do not exceed the shelf life of the components. After thawing aliquots, use immediately. Do not re-freeze.
2. Add 20 mL of STEMdiff™ Myogenic Progenitor Supplement D to 80 mL DMEM/F-12 (without HEPES). Mix thoroughly. Warm to room temperature before use.

   NOTE: If not used immediately, store at 2 - 8°C for up to 4 weeks.

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 Human Pluripotent Stem Cells (hPSCs)

B. Culture of Single-Cell hPSCs

C. Myogenic Differentiation (Day 0 - 29)

D. Sub-Culturing hPSC-Myogenic Progenitor Cells (hPSC-MPs) (Day 30)

E. Differentiation of hPSC-MPs to Myotubes

A. DISSOCIATION OF HUMAN PLURIPOTENT STEM CELLS (hPSCs)

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 differentiation into myogenic progenitor cells. hPSCs must have high expression of markers such as OCT4 and TRA-1-60.

For complete instructions on maintaining hPSCs in mTeSR™1, mTeSR™ Plus, or TeSR™-E8™, 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 6-well tissue culture-treated plate with Corning® Matrigel®. Warm to room temperature (15 - 25°C) for at least 1 hour prior to use.
2. Wash each well of hPSCs to be passaged with 1 mL D-PBS (Without Ca++ and Mg++).
3. Aspirate D-PBS and add 1 mL Gentle Cell Dissociation Reagent per well.
4. Incubate at 37°C and 5% CO_2 for 8 - 10 minutes.
5. In each well, use a pipettor with a 1 mL tip to dislodge cells by pipetting up and down 3 - 4 times.
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 in 1 - 2 mL of mTeSR™1, mTeSR™ Plus, or TeSR™-E8™ + 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

The following instructions are for one well of a 6-well plate. For other cultureware, adjust volumes accordingly.

1. Day -1: Coat a tissue culture-treated 6-well plate with Corning® Matrigel®. Add 2 mL mTeSR™1, mTeSR™ Plus, or TeSR™-E8™ supplemented with 10 µM Y-27632 per well.
2. Add hPSCs (prepared in section A) at a density of 30,000 - 40,000 cells/cm^2. 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. Optimization of cell density may be required.
3. Incubate at 37°C for 24 hours. Do not disturb cells.
4. Day 0: Assess cells for confluency. Colonies must contain approximately 10 - 30 cells after 24 hours of incubation and before starting the differentiation protocol (section C). Refer to Figure 1 for a representative image of the confluency of a starting culture.

   NOTE: If required, repeat steps 1 - 4 (section B) and adjust hPSC seeding density to obtain optimal confluency.

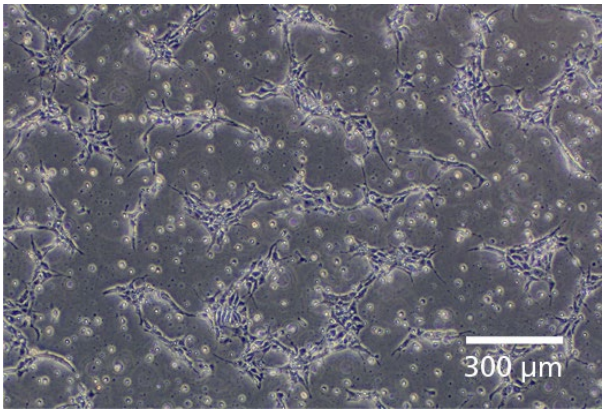


FIGURE 1. Day 0 hPSC Culture

C. MYOGENIC DIFFERENTIATION (DAY 0 - 29)

The following instructions are for one well of the 6-well plate from section B. For other cultureware, adjust volumes accordingly.

Perform a full-medium change daily on Day 0 - 29 by removing the medium from the well and adding 2 mL of fresh medium as indicated in Table 1. Incubate at 37°C and 5% CO_2. Assess culture morphology throughout the protocol.

Table 1. Media for Daily Full-Medium Changes on Day 0 - 29

DAY	MEDIUM
0	STEMdiff™ Myogenic Progenitor Medium A
1	STEMdiff™ Myogenic Progenitor Medium A
2	STEMdiff™ Myogenic Progenitor Medium B
3	STEMdiff™ Myogenic Progenitor Medium B
4	STEMdiff™ Myogenic Progenitor Medium C
5	STEMdiff™ Myogenic Progenitor Medium C
6 - 29	STEMdiff™ Myogenic Progenitor Medium D

Day 2: Assess cells for confluency. Cultures should be approximately > 80% confluent. Refer to Figure 2 for a representative example of the required confluency.

NOTE: If cells are < 80% confluent, do not continue protocol; repeat section B steps 1 - 4 and adjust hPSC seeding density to obtain optimal confluency.

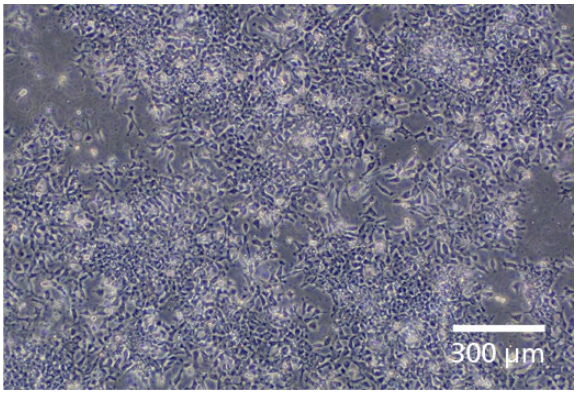


FIGURE 2. Day 2 Culture of Myogenic Differentiation

Day 6: Formation of 3D structures should be present (Figure 3A).

Day 6 - 30: Cells should develop into a dense, multi-layered, and tightly packed culture (Figures 3B and C). Medium will turn yellow during later stages of the culture period (Day 10 onward).

NOTE: Variation in morphology of cultures among hPSC lines may be observed.

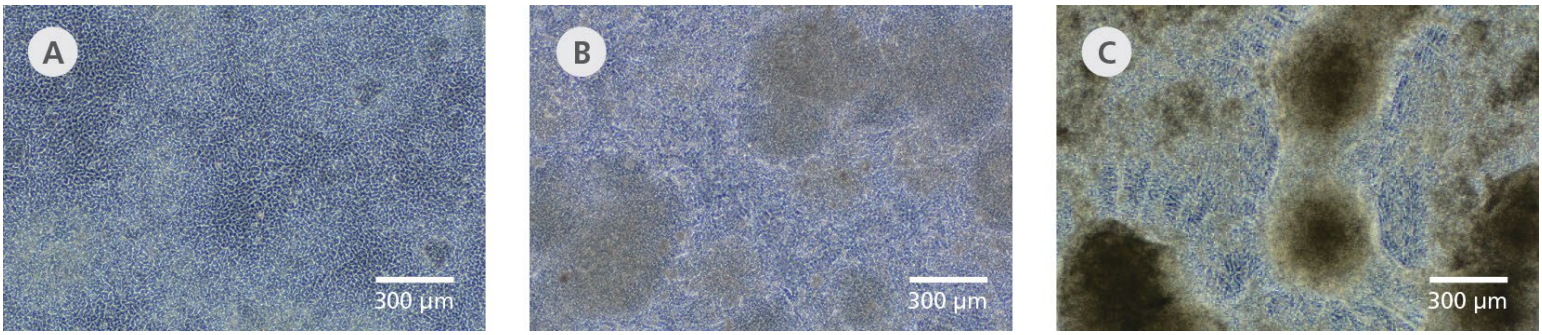


FIGURE 3. Cultures of Myogenic Differentiation on (A) Day 6, (B) Day 10, and (C) Day 30

On Day 30, proceed to section D for dissociation and sub-culturing.

D. SUB-CULTURING hPSC-MYOGENIC PROGENITOR CELLS (hPSC-MPs) (DAY 30)

The following instructions are for one well of a 6-well plate. For other cultureware, adjust volumes accordingly.

1. Day 30: Remove medium from the 6-well plate from section C. Gently rinse culture with 2 mL of D-PBS (Without Ca++ and Mg++) per well. Remove and discard D-PBS.
2. Add 0.5 mL/well TrypLE™ Express. Incubate at 37°C and 5% CO_2 for 6 minutes.
3. Add 200 µL/well Collagenase Type IV. Using a sterile 1 mL cut pipette tip (for increased tip diameter), gently triturate culture by pipetting up and down for 2 minutes. Incubate at 37°C and 5% CO_2 for 4 minutes.
4. Add 3 mL/well DMEM/F-12. Continue to triturate culture by pipetting for 1 - 2 minutes with the cut 1 mL pipette tip.
5. Using a 5 mL serological pipette, transfer the cell suspension to a 70 µm Reversible Strainer on a 50 mL conical tube. Wash the well with an additional 1 mL of DMEM/F-12 and transfer the wash to the strainer.
6. Transfer the flow-through to a 15 mL conical tube and centrifuge at 300 x *g* for 5 minutes. Remove supernatant carefully with a Pipette-Aid or pour off without disturbing the pellet. Discard supernatant.
7. Prepare MyoCult™-SF Expansion Medium as directed in the Product Information Sheet (PIS) for MyoCult™-SF Expansion Supplement Kit (Human).
8. Add 400 µL of MyoCult™-SF Expansion Medium + 10 µM Y-27632 to the cell pellet from step 6. Mix gently to resuspend the pellet.
9. Perform a cell count using Trypan Blue and a hemocytometer.

   NOTE: Expected cell yield is approximately 1 - 4 x 10^6 cells/well.
10. Add cells to a Corning® Matrigel®-coated plate at a density of 15,000 - 20,000 cells/cm^2 in MyoCult™-SF Expansion Medium + 10 µM Y-27632. Incubate at 37°C and 5% CO_2 for 24 hours.
11. Perform a full-medium change with fresh MyoCult™-SF Expansion Medium (without Y-27632). Incubate at 37°C and 5% CO_2.
12. When the culture is 60 - 80% confluent, it can be harvested and cryopreserved, or counted and replated for further expansion and/or differentiation experiments (section E). Harvest cells using Animal Component-Free Cell Dissociation Kit, then proceed as follows:
    - For expansion and further passaging, seed cells onto a Corning® Matrigel®-coated plate at 5000 - 10,000 cells/cm^2 in MyoCult™-SF Expansion Medium. Use Animal Component-Free Cell Dissociation Kit for passaging.
    - For cryopreserving cells, use CryoStor® CS10 (Catalog #07930).
    - For differentiation, proceed to section E.

E. DIFFERENTIATION OF hPSC-MPs TO MYOTUBES

The following instructions are for a 12-well tissue culture-treated plate; if using other cultureware, adjust accordingly.

1. Add hPSC-MPs (from section D) at 80,000 cells/well (20,000 cells/cm^2) in MyoCult™-SF Expansion Medium on a Matrigel®-coated plate. Incubate at 37°C and 5% CO_2.
2. Perform a full-medium change with MyoCult™-SF Expansion Medium every other day. When culture is 95 - 100% confluent, proceed to step 3.
3. Aspirate medium. Wash cells with D-PBS (Without Ca++ and Mg++); remove and discard D-PBS.
4. Prepare MyoCult™ Differentiation Medium as directed in the PIS for MyoCult™ Differentiation Kit (Human).
5. Add 1.5 mL of MyoCult™ Differentiation Medium per well. Incubate at 37°C and 5% CO_2 for 5 days.
6. Perform a half-medium change by removing 0.75 mL of medium and adding 0.75 mL of fresh MyoCult™ Differentiation Medium. Incubate at 37°C and 5% CO_2 for up to 5 days. Once myotubes have formed, cells can be used in downstream applications.

Related Products

For related products, including antibodies, small molecules, and cultureware, visit www.stemcell.com/myogenicworkflow, or contact us at techsupport@stemcell.com.