STEMdiff™ Cardiomyocyte Differentiation and Maintenance Kits

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Media for differentiation of human PSCs to cardiomyocytes and long-term maintenance of human PSC-derived cardiomyocytes

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 x 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.

<table>
<thead>
<tr>
<th>COMPONENT NAME</th>
<th>COMPONENT #</th>
<th>SIZE</th>
<th>STORAGE</th>
<th>SHELF LIFE</th>
</tr>
</thead>
<tbody>
<tr>
<td>STEMdiff™ Cardiomyocyte Differentiation Kit (05010)</td>
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<tr>
<td>STEMdiff™ Cardiomyocyte Differentiation Basal Medium</td>
<td>05011</td>
<td>380 mL</td>
<td>Store at 2 - 8°C.</td>
<td>Stable for 12 months from date of manufacture (MFG) on label.</td>
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<tr>
<td>STEMdiff™ Cardiomyocyte Differentiation Supplement A (10X)*</td>
<td>05012</td>
<td>10 mL</td>
<td>Store at -20°C.</td>
<td>Stable for 2 years from date of manufacture (MFG) on label.</td>
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<tr>
<td>STEMdiff™ Cardiomyocyte Differentiation Supplement B (10X)*</td>
<td>05013</td>
<td>10 mL</td>
<td>Store at -20°C.</td>
<td>Stable for 2 years from date of manufacture (MFG) on label.</td>
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<tr>
<td>STEMdiff™ Cardiomyocyte Differentiation Supplement C (10X)*</td>
<td>05014</td>
<td>20 mL</td>
<td>Store at -20°C.</td>
<td>Stable for 2 years from date of manufacture (MFG) on label.</td>
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<tr>
<td>STEMdiff™ Cardiomyocyte Maintenance Basal Medium</td>
<td>05015</td>
<td>490 mL</td>
<td>Store at 2 - 8°C.</td>
<td>Stable for 12 months from date of manufacture (MFG) on label.</td>
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<tr>
<td>STEMdiff™ Cardiomyocyte Maintenance Supplement (50X)*</td>
<td>05016</td>
<td>10 mL</td>
<td>Store at -20°C.</td>
<td>Stable for 2 years from date of manufacture (MFG) on label.</td>
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*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

<table>
<thead>
<tr>
<th>PRODUCT NAME</th>
<th>CATALOG #</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corning® Matrigel® hESC-Qualified Matrix</td>
<td>Corning 354277</td>
</tr>
<tr>
<td>mTeSR™1 OR TeSR™-E8™</td>
<td>85850 OR 05990</td>
</tr>
<tr>
<td>D-PBS (Without Ca++ and Mg++)</td>
<td>37350</td>
</tr>
<tr>
<td>Gentle Cell Dissociation Reagent</td>
<td>07174</td>
</tr>
<tr>
<td>Y-27632</td>
<td>72302</td>
</tr>
<tr>
<td>Trypan Blue</td>
<td>07050</td>
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</table>
**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.

   
   **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.

   
   **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**

```
Day   | 0 | 2 | 4 | 6 | 8 | 15

Medium | mTeSR™1 or TeSR™-E8™ |  |  |  |  |  | STEMdiff™ Cardiomyocyte Maintenance Medium (Every 2 days)

Day 2: hPSC Monolayer (95% Confluent) | Medium A | Medium B | Medium C | Medium C

Day 0: hPSC-Derived Cardiomyocytes
```

**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 10^5 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.