Protocol for hPSC Differentiation to Skeletal Muscle

This protocol describes conversion of human pluripotent stem cells to skeletal muscle myotubes, through myogenic precursor (Pax7) and myoblast (MyoD) transitions.

![Diagram](image)

**Figure 1. Genea Biocells Skeletal Muscle differentiation as per Caron et. al. 2016. Schematic of differentiation.**

### Reagents:

**SKM-KITM - Skeletal Muscle Differentiation Kit**

- provided by Genea Biocells comprises:
  - SKM-01 | 250mL Skeletal Muscle Induction Medium (Stage I)
  - SKM-02 | 250mL Skeletal Myoblast Medium (Stage II)
  - SKM-03 | 250mL Myotube Medium (Stage III, Option 1)
  - SKM-03+ | 250mL Myotube Fusion Medium (Stage III, Option 2)

**Collagen I coated plates** - We recommend using the BD Biocoat™ Collagen I range of pre-coated plates and flasks for best performance. Alternatively, plates can be self-coated with Collagen I solution (Gibco).

**0.05% Trypsin-EDTA** - Mixed from commercially available solutions.

The differentiation is performed at 37 degrees Celsius in 5% CO₂/20% O₂.

### Approximate media volumes:

<table>
<thead>
<tr>
<th>Plate/Flask Format</th>
<th>Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>96 well plate</td>
<td>100μL/well</td>
</tr>
<tr>
<td>24 well plate</td>
<td>0.5mL/well</td>
</tr>
<tr>
<td>12 well plate</td>
<td>1 to 2 mL</td>
</tr>
<tr>
<td>6 well plate</td>
<td>3 to 4 mL</td>
</tr>
<tr>
<td>T-25 Flask</td>
<td>7mL to 8mL</td>
</tr>
<tr>
<td>T-75 Flask</td>
<td>15mL to 20mL</td>
</tr>
<tr>
<td>T-175 Flask</td>
<td>35mL to 50mL</td>
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</tbody>
</table>
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2. Ordering Information
Differentiation Protocol

Stage I - Conversion of human PSC to myogenic precursors

1.1. Myogenic precursor induction from human Pluripotent Stem Cells
   1.1.1. Dissociate mTESR hESC with Accutase for ≈5 min at 37°C
   1.1.2. Harvest cells in Stage I media.
   1.1.3. Centrifuge the cells for 4 mins at 1200 rpm.
   1.1.4. Aspirate media without disturbing the cell pellet and resuspend cells in warm Skeletal Muscle Induction Medium.
   1.1.5. Count the number of viable cells.
   1.1.6. Plate the cells at 5000 cells/cm² onto a collagen I coated plate.
       **Note:** Successful skeletal muscle differentiation has been observed in difficult cell lines, plated at densities from 2500 to 10,000 cells/cm².
   1.1.7. Perform a media change every 2 with Skeletal Muscle Induction Medium.
   1.1.8. Cells are maintained for 6-10 days in Skeletal Muscle Induction Medium or until confluent.
   1.1.9. Use for assay requirements, or continue to next stage.

1.2. Representative image of pluripotent stem cell to myogenic precursor transition

<table>
<thead>
<tr>
<th>hESC / iPSc Line</th>
<th>Stage I</th>
<th>Myogenic Precursor</th>
</tr>
</thead>
</table>

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Stage II - Conversion of Myogenic Precursors to Myoblasts

1.3. Conversion of Myogenic Precursors to Myoblasts

1.3.1. After 6-10 days in Skeletal Muscle Induction Medium, dissociate myogenic precursors with 0.05% Trypsin-EDTA for 5 min at 37°C.
1.3.2. Resuspend the cells with medium containing 10% serum.
1.3.3. Centrifuge the cells for 4 mins at 1200 rpm.
1.3.4. Aspirate media without disturbing the cell pellet and resuspend cells in warm Skeletal Myoblast Medium.
1.3.5. Count the number of viable cells.
1.3.6. Plate the cells at 5000 cells/cm² onto a collagen I coated plate.
1.3.7. Perform a media change every 2 days with Skeletal Myoblast Medium.
1.3.8. Myoblasts are maintained for 6-8 days in Skeletal Myoblast Medium or until the cells reach confluence.
1.3.9. Use for assay requirements, or continue to next stage.

1.4. Representative image of myogenic precursor to myoblast transition

<table>
<thead>
<tr>
<th>Myogenic Precursor</th>
<th>Stage II</th>
<th>Myoblasts</th>
</tr>
</thead>
</table>

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HUMANLY POSSIBLE
Stage III – Differentiation of Myoblasts to post mitotic Myotubes

1.5. Conversion of Myoblasts to Skeletal Muscle Myotubes

1.5.1. If Continuing Cultured Cells From step 1.3.9

1.5.1.1. When myoblasts are confluent from Stage II (usually 6-8 days) switch the media to either Myotube Medium or Myotube Fusion Medium.

Note:

1) Myoblasts are not dissociated at this stage; ensure the cells have formed a confluent monolayer to fully differentiate into myotubes.

2) Use of Myotube/Myotube Fusion Medium will depend on end user assay requirements.

1.5.1.2. Perform a media change every 3 to 4 days with Myotube/Myotube Fusion Medium.

1.5.2. If Using Myoblast Cells provided by Genea Biocells

1.5.2.1. Prepare a 15mL Falcon tube with 3mL warm Skeletal Myoblast Medium for each vial thawed.

1.5.2.2. Remove vial from LN2 storage and thaw in a 37°C water bath.

1.5.2.3. As soon as the vial is thawed, use a 1mL pipette to slowly transfer cells to the warm Skeletal Myoblast Medium.

1.5.2.4. Mix by gentle inversion.

1.5.2.5. Centrifuge the cells for 4 mins at 1200 rpm.

1.5.2.6. Aspirate media without disturbing the cell pellet and resuspend cells in 5mL warm Skeletal Myoblast Medium.

1.5.2.7. Assess the number of viable cells.

1.5.2.8. Plate at 30,000 – 60,000 cells/cm²

1.5.2.9. Change media every 2 days with Skeletal Myoblast Medium.

1.5.2.10. When confluent (usually 3-4 days) change the media to Myotube Medium or Myotube Fusion Medium.

1.5.3. Representative image of myoblast to myotube transition

- [Image: Myoblasts]
- [Image: Stage III]
- [Image: Myotubes in Myotube Fusion Medium]
### 1.5.4. Comparison between Myotube Medium and Myotube Fusion Medium

<table>
<thead>
<tr>
<th>Genea002</th>
<th>Genea019</th>
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<tbody>
<tr>
<td><img src="image1.png" alt="Image" /></td>
<td><img src="image2.png" alt="Image" /></td>
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<td><img src="image3.png" alt="Image" /></td>
<td><img src="image4.png" alt="Image" /></td>
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<table>
<thead>
<tr>
<th>Stage 3</th>
<th>Myotubes in SkM. Myotube Medium (SKM-03)</th>
<th>Myotubes in SkM. Myotube Fusion Medium (SKM-03+)</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image5.png" alt="Image" /></td>
<td><img src="image6.png" alt="Image" /></td>
<td><img src="image7.png" alt="Image" /></td>
</tr>
</tbody>
</table>

- **Genea019**
  - **MyoG**: 1:200
  - **MHC**: 1:1000
## 2. Ordering information

<table>
<thead>
<tr>
<th>Cat #</th>
<th>Description</th>
</tr>
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<tbody>
<tr>
<td>SKM01-250mL</td>
<td>SkM. Induction Medium, 250mL Bottle</td>
</tr>
<tr>
<td>SKM02-250mL</td>
<td>SkM. Myoblast Medium, 250mL Bottle</td>
</tr>
<tr>
<td>SKM03-250mL</td>
<td>SkM. Myotube Medium, 250mL Bottle</td>
</tr>
<tr>
<td>SKM03+-250mL</td>
<td>SkM. Myotube Fusion Medium, 250mL Bottle</td>
</tr>
<tr>
<td>G***SKM</td>
<td>Vial of Genea Myoblasts (~3x10^6/vial) (where *** is cell line number)</td>
</tr>
</tbody>
</table>