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Datasheet

Skeletal Muscle Cells Culture Protocols

Product Information

Catalog Number **ASM-6007 (Muscle Cellulations Medium)**

Application The cell culture protocol provided in this manual are for *in vitro* culture protocols of human skeletal muscle cells using optimized reagents. Changes in the experimental conditions may potentially addect cell survival and/or yield abnormal cell growth.

Materials Required

Materials	Equipment
• Cells ASE-5015, ASE-5065, ASE-5203 • Muscle Cellutions Media (ASM-6007) • Fibroblast Growth Factor-2 (FGF-2, basic FGF, bFGF; final concentration – 10 ng/mL) • Collagen coating solution (150 µg/mL) • Fetal Bovine Serum (FBS) • Tissue culture vessels (T25 flask, 6 or 12 well plates) • 1X PBS (Ca2+/Mg2+-free) • Trypsin or equivalent • Dimethyl Sulfoxide (DMSO)	• Biosafety Cabinet • Incubator, 37°C/5% CO2 • Water Bath 37°C • Centrifuge • Pipette

Protocol

1. **Immediately Upon Delivery**
   1.1 Remove vial from shipping container to check that it is still frozen.
   1.2 Transfer frozel vial to liquid nitrogen until you are ready to thaw and begin cell culture.
   1.3 Prior to thawing cells, make sure all reagents and culture vessels are ready to use.
   1.4 Coat cell culture vessels with collagen coating solution (150 µg/mL). Incubate at room temperature for 1 hour. Aspirate collagen coating. Wash the culture vessel with PBS.

2. **Thawing Cells**
   2.1 Perform and maintain cell culture using aseptic techniques.
   2.2 Aliquot and warm only required volume of muscle cellutions media (basal + supplement). Add fresh FGF-2 (10 ng/mL) to prepare complete muscle cellutions medium.
   2.3 Thaw cells rapidly and with agitation.
   2.4 Centrifuge at 400 g for 5 minutes. Remove supernatant and resuspend cell pellet in 1-5 mL fresh 37°C warm Muscle Cellutions Media with FGF-2 (freshly prepared complete muscle cellutions medium).
   2.5 Count cells and add complete medium to adjust the cell suspension volume as required for appropriate seeding density.

3. **Cultivating Cells**
   3.1 Seed cells at a density of 5.0×10^3 cells/ cm^2 – 1.0×10^4 cells/cm^2. Carefully add cell suspension to the collagen-coated culture vessel.
   3.2 Leave cells undisturbed for 2 days, allowing them to attach. Feed cells on the third day by completely replacing spent medium with a fresh volume of 37°C warm complete medium.
   3.3 Observe cells on a daily basis.

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3.4 Feed cells every 2-3 days by performing a complete medium change as described above.
3.5 When cells have reached 60-70% confluency, feed at the more regular frequency of every other day.
3.6 When cells approach approximately 80% confluency, prepare culture vessels for passage, expansion or use.

Note: Do not let cells become overly confluent in the culture vessel.

4. **Passing and Sub-cultivation of Cells**

4.1 Aspirate and discard medium; wash cells with PBS (Ca2+/Mg2+ free).
4.2 Aspirate PBS. Add trypsin or equivalent enzyme solution.
4.3 Incubate for 5 minutes at 37°C. Monitor cells to observe for detachment.

Note: When using trypsin, closely monitor the detachment of cells every 1-2 minutes in order to avoid toxic effects.

4.4 Add muscle cellutions medium or FBS immediately to detached cell to stop enzyme. Transfer cell suspension to a centrifuge tube.
4.5 Wash flasks/plate with warm PBS (with a volume equal to that of the enzyme solution used) and collect any remainder cells. Add the washed material to the centrifuge tube.
4.6 Centrifuge the collected cell mixture at 400 g at 4°C for 5 minutes, and aspirate supernatant
4.7 Resuspend cells in fresh complete muscle cellutions medium for counting. Prepare cell suspension volume at the appropriate seeding density.
4.8 Seed cells in vessel, pre-coated with collagen coating solution as describe in the previous section, at the cell seeding density of 5.0×10^3 cells/ cm^2 – 1.0×10^4 cells/cm^2.
4.9 If freezing cells for storage:

- Resuspend cell pellet in cold FBS
- Remove aliquot and perform cell count
- Place cells on ice
- Determine how many vials to freeze (recommended $1.0 \text{x} 10^6$ to no more than $5.0 \text{x} 10^6$ in 1mL per vial.
- Slowly and dropwise add equal volume cold FBS containing 20% (v/v) dimethyl sulfoxide (DMSO). This achieves a final volume of 10% (v/v) dimethyl sulfoxide (DMSO).
- Immediately remove vials to liquid nitrogen and place at -80°C or liquid nitrogen.