



Human Skeletal Muscle Cells



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Human Skeletal Muscle Cells

| Catalog No. | Product Name | Product Quantity | Short-term Storage | Long-term Storage | Thawing Instructions |
|-------------|---|---------------------------|--------------------------------|---|------------------------------------|
| ax3051 | Human Skeletal Muscle Cells (Adult) | 500,000 cells/vial | Liquid Nitrogen | Liquid Nitrogen | See below |
| ax3055 | Human Skeletal Muscle Cells – Duchenne Muscular Dystrophy Patient | 500,000 cells/vial | Liquid Nitrogen | Liquid Nitrogen | See below |
| ax3060 | Skeletal Muscle Cell Culture Medium | 500 mL | Store at 4°C for up to 1 month | Aliquot and store at -20°C for up to 6 months | Thaw at 4°C or at room temperature |
| ax0047 | Recombinant Human FGF2 | 100 µg Lyophilized Powder | -20°C | Reconstituted protein should be used immediately or stored in working aliquots at -20°C | N/A |

Lot-specific information such as donor details and passage number are stated in the Certificate of Analysis for each product.

Recommendations

- Recommended culture vessel coating: **Collagen (150 µg/ml)**
- Recommended cell culture medium: **Skeletal Muscle Cell Culture Medium**
- Recommended seeding density: **10,000 viable cells/cm²**
- Recommended centrifugation speed: **400 x g for 5 minutes**
- Frequency of media changes: Every 2-3 days depending on cell confluency

Preparation of Reagents

Recombinant Human FGF2 Reconstitution

- Prepare **100 µg/mL** solution (5000x) of **Recombinant Human FGF2** by resuspending the 100 µg of lyophilized powder in 1 mL of PBS (1x) supplemented with 0.1 % human serum albumin.

Skeletal Muscle Cell Culture Medium

- Upon receipt aliquot and store at **-20°C**. Stored at **-20°C**, medium is stable for 6 months from date of manufacture..
- Prior to use, **Skeletal Muscle Cell Culture Medium** requires supplementation to a final concentration of **10 ng/mL Recombinant Human FGF2** to yield the complete growth medium.
- The growth factor should be **added fresh each time** an aliquot of **Skeletal Muscle Cell Culture Medium** is thawed.

Coating

- Coat the cell culture vessels with Collagen coating solution (**150 µg/ml**), following the supplier's instructions, or use pre-coated culture vessels.
- Aspirate Collagen from the culture vessel and wash with 1x PBS before seeding the cells.

Culturing Human Skeletal Muscle Cells

Thawing and Plating

- Transfer the cells from liquid nitrogen storage with the cells buried in dry ice. Remove the cells from dry ice and transfer them immediately to a **37°C** water bath.
- Thaw the cells quickly in a **37°C** water bath. Remove the vial before the last bit of ice has melted, after **~1-2 minutes**.
- Wipe the outside of the vial with 70% ethanol.
- Gently resuspend the cells and transfer to a 15 mL sterile conical tube.
- Slowly add 10 mL of pre-warmed **Skeletal Muscle Cell Culture Medium**.
- Rinse the cryovial with 1 mL of **Skeletal Muscle Cell Culture Medium** to ensure all of the cells are transferred.
- Centrifuge the cells at **400 x g** for **5 minutes**.
- Carefully remove the supernatant and resuspend the cell pellet in 1 mL of pre-warmed **Skeletal Muscle Cell Culture Medium** freshly supplemented with **10 ng/mL Recombinant Human FGF2**.
- Perform a cell count to determine the number of viable cells.
- Dilute the cells into the required volume of pre-warmed **Skeletal Muscle Cell Culture Medium** freshly supplemented with **10 ng/mL Recombinant Human FGF2**.
- Seed cells into the Collagen coated culture vessel at the recommended seeding density of **10,000 viable cells/cm²**.
- Incubate the cells at **37°C, 5% CO₂** in a humidified incubator.
- Leave the cells undisturbed for **2 days**, allowing them to attach. On **day 3** after seeding, completely replace the culture medium with fresh, pre-warmed **Skeletal Muscle Cell Culture Medium** freshly supplemented with **10 ng/mL Recombinant Human FGF2**.
- Observe the cells on a daily basis to assess confluency and cell health.
- Frequency of media changes: **Every 2-3 days** depending on cell confluency

Note:

There may be a significant number of unattached cells. These can be collected, centrifuged and re-seeded into the same vessel for maximal recovery.

Passaging

- **Passage when the culture reaches:** **80% confluent**
- **Recommended passaging reagent:** **Trypsin-EDTA**
- After adding passaging reagent, incubate the cells for **5 minutes** at **37°C**. Observe the cells at regular intervals for detachment from the culture vessel.
- Once the cells have detached, neutralize the trypsin with pre-warmed, **37°C Skeletal Muscle Cell Culture Medium**.
- Centrifuge the cells at **400 x g** for **5 minutes**.
- Remove the supernatant and resuspend the cell pellet in **1-2 mL** of pre-warmed **Skeletal Muscle Cell Culture Medium** freshly supplemented with **10 ng/mL Recombinant Human FGF2**.
- Perform a cell count to determine the number of viable cells.
- Dilute the cells into the required volume of pre-warmed **Skeletal Muscle Cell Culture Medium** freshly supplemented with **10 ng/mL Recombinant Human FGF2**.
- Seed cells into the Collagen coated culture vessel at the recommended seeding density of **10,000 viable cells/cm²**.
- Incubate the cells at **37°C, 5% CO₂** in a humidified incubator.

Usage Statement

Our products are intended for research use only and are not to be used for any other purpose, which includes but is not limited to, unauthorized commercial uses, *in vitro* diagnostic uses, *ex vivo* or *in vivo* therapeutic uses or any type of consumption or application to humans.

Got any questions? Need help with the protocol?
Contact Axol Technical Support at support@axolbio.com
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Notes

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