Human Skeletal Muscle Cells

Catalog No.	Product Name	Product quantity	Short-term Storage	Long-term Storage	Thawing Instructions
ax3050	Human Skeletal Muscle Progenitor Cells (Adult)	500,000 cells/vial	Liquid Nitrogen	Liquid Nitrogen	See below
ax3051	Human Skeletal Muscle Cells (Adult)	500,000 cells/vial	Liquid Nitrogen	Liquid Nitrogen	See below
ax3054	Human Skeletal Muscle Progenitor Cells – Duchenne Muscular Dystrophy Patient	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	SureGrowth Recombinant Human FGF2	100 µg Lyophilized Powder	-20°C	Reconstituted protein should be used immediately or stored in working aliquots at -20°C	Thaw aliquots at 4°C or at room temperature
ax3061	Skeletal Muscle Differentiation 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

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**
- Recommended cell culture medium: **Skeletal Muscle Cell Culture Medium** followed by **Skeletal Muscle Differentiation Medium**
- Recommended seeding density: **10,000 viable cells/cm^2**
- Recommended centrifugation speed: **400 x *g*** for **5 minutes**
- Frequency of media changes: **Every 2-3 days** depending on cell confluency

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Coating:

- Coat the cell culture vessels with **collagen**, following the supplier's instructions, or use pre-coated culture vessels.

Skeletal Muscle Cell Culture Medium:

- The culture medium should be aliquoted in suitable volumes and stored at -20°C for up to 6 months.
- Thaw an aliquot overnight at 4°C or at room temperature.
- Prior to use, **Skeletal Muscle Cell Culture Medium** requires supplementation with **20 ng/mL SureGrowth Recombinant Human FGF2** to yield the complete growth medium.

- **SureGrowth Recombinant Human FGF2 Reconstitution:**
  - Prepare **100 µg/mL** solution (5000x) of **SureGrowth 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 Differentiation Medium:

- The differentiation medium is fully supplemented and ready to use. The medium should be aliquoted in suitable volumes and stored at -20°C for up to 6 months.

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Thawing & Plating:

- Transfer the vial of cells from liquid nitrogen storage with the vial buried in dry ice. Remove the vial from dry ice and transfer it immediately to a **37°C** water bath.
- Thaw the cells quickly in a **37°C** water bath until just prior to complete thawing.
- 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** supplemented with **20 ng/mL SureGrowth 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** supplemented with **20 ng/mL SureGrowth Recombinant Human FGF2**.
- Seed cells into the collagen-coated culture vessel at the recommended seeding density of **10,000 viable cells/cm^2**.
- Incubate the cells at **37°C**, **5% CO_2** in a humidified incubator.
- Leave the cells undisturbed for 2 days. On **day 3** after seeding, replace the culture medium with fresh, pre-warmed **Skeletal Muscle Cell Culture Medium** supplemented with **20 ng/mL SureGrowth Recombinant Human FGF2**.
- Replace the culture medium **every 2-3 days** depending on cell confluency.
- Observe the cells on a daily basis to assess confluency and cell health.

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.

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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** supplemented with **20 ng/mL SureGrowth 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** supplemented with **20 ng/mL SureGrowth Recombinant Human FGF2**.
- Seed cells into the collagen-coated culture vessel at the recommended seeding density of **10,000 viable cells/cm^2**.
- Incubate the cells at **37°C**, **5% CO_2** in a humidified incubator.
- Replace the culture medium **every 2-3 days** depending on cell confluency.
- Observe the cells on a daily basis to assess confluency and cell health.

Terminal Differentiation:

- Allow the cells to reach confluency in the collagen-coated culture vessels required for endpoint assays.
- Once confluent, replace the culture medium with a 50:50 mix of **Skeletal Muscle Cell Culture Medium** (**without FGF2 added**) and **Skeletal Muscle Differentiation Medium**.
- Allow the cells to adjust to the new medium for a minimum of **24 h**.
- After 24 h (**Day 1**), replace the culture medium with fresh, pre-warmed **Skeletal Muscle Differentiation Medium**.
- On **Day 2** and **Day 3**, replace the culture medium with fresh, pre-warmed **Skeletal Muscle Differentiation Medium**.
- Observe the cells on a daily basis to assess cell health.
- Replace the culture medium **every 3 days**.
- Leave the cells in **Skeletal Muscle Differentiation Medium** for a minimum of **2 weeks** before conducting endpoint assay experiments.
- Terminally differentiated myoblasts should be visible by **Day 14**.

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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**
International phone **+44-1223-751-051**
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