Human Skeletal Muscle Cells

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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	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**
- 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**

Preparation of Reagents

Skeletal Muscle Cell Culture 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.
- Prior to use, **Skeletal Muscle Cell Culture Medium** requires supplementation with **20 ng/mL SureGrowth 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, following the supplier's instructions, or use pre-coated culture vessels.

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 **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** freshly supplemented with **20 ng/mL SureGrowth Recombinant Human FGF2**.
- Seed cells into the culture vessel at the recommended seeding density of **10,000 viable cells/cm²**.
- Incubate the cells at **37°C**, **5% CO_2** in a humidifled 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** freshly supplemented with **20 ng/mL SureGrowth Recombinant Human FGF2**.
- Observe the cells on a daily basis to assess con‡uency 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 **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** freshly supplemented with **20 ng/mL SureGrowth Recombinant Human FGF2**.
- Seed cells into the culture vessel at the recommended seeding density of **10,000 viable cells/cm²**.
- Incubate the cells at **37°C**, **5% CO_2** in a humidifled 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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