axol

Human Skeletal Muscle Progenitor 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
ax3054	Human Skeletal Muscle Progenitor 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 **-20ºC**. Stored at **-20ºC**, 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 **20 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 Progenitor 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 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 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% CO_2** 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 **20 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, feed every other day once reached 60-70% 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** at **4°C**.
- 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 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 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% CO_2** in a humidified incubator.

Terminal Differentiation

- Seed cells into the Collagen coated culture vessel in **Skeletal Muscle Cell Culture Medium** freshly supplemented with **20 ng/mL Recombinant Human FGF2** at the recommended seeding density of **10,000 viable cells/cm²**.
- Once confluent, feed the cells with 50:50 mix of **Skeletal Muscle Cell Culture Medium** (without **Recombinant Human FGF2**) and **Skeletal Muscle Cell Differentiation Medium**.
- Culture the cells for a minimum of **24 hours**. This is day 0.
- The following day (day 1) feed the cells with a complete medium change to S**keletal Muscle Cell Differentiation Medium**.
- Repeat the above step on **day 2** and **day 3**, preparing fresh media each time.

Note:

Observe cells regularly for lifting and toxicity.

- Culture the cells in **Skeletal Muscle Cell Differentiation Medium** for a minimum of **2 weeks**, changing the media **every 2-3 days**.

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**
US phone **+1-800-678-AXOL (2965)**

Notes

Address

Axol Bioscience Limited | Suite 3 | The Science Village |
Chesterford Research Park | Little Chesterford | Cambridgeshire | CB10 1XL

International phone

+44-1223-751-051

US phone

+1-800-678-AXOL (2965)

Email

support@axolbio.com

Web

www.axolbio.com