www.ixcellsbiotech.com
7270 Trade Street, Suite 102, San Diego, CA 92121
Tel: (858)412-5988 Fax: (858)368-8716
Technical Supports: supports@ixcellsbiotech.com
Orders: orders@ixcellsbiotech.com

Product Information

Mouse Skeletal Muscle Myoblasts (MSkMM)

Catalog Number	10MU-033	**Cell Number**	0.5 million cells/vial
Species	*Mus musculus*	**Storage Temperature**	Liquid Nitrogen

Description

Myoblast is a type of progenitor cells that differentiates to give rise to muscle cells [1]. They are the main effector cells in the myogenesis by fusing with each other to injured myofibers leading to the formation of new myofibers or regenerations of skeletal muscle in adults [2]. Many steps of myogenesis can be recapitulated through *in vitro* differentiation of myoblasts into myotubes. Primary myoblasts have been suggested as the most physiologically relevant model for studying myogenesis *in vitro* [3].

MSkMM from iXCells are derived from mouse skeletal satellite cells isolated from mouse gastrocnemius (GA) and tibialis anterior (TA) muscles of both hind limbs by culturing them on matrigel-coated plastic dishes (Figure 1). MSkMM are cryopreserved at passage one and delivered frozen. Each vial contains ≥5 x 10^5 cells/vial. MSkMM are characterized by immunofluorescence with antibodies specific to MyoD and Desmin (Figure 2). MSkMM can be in vitro differentiated into myotube using Mouse Skeletal Muscle Myoblast Differentiation Medium (Cat# MD-0065). MSkMM are guaranteed to further expand two passages using the culture medium provided by iXCells. These cells are negative for mycoplasma, bacteria, yeast and fungi.

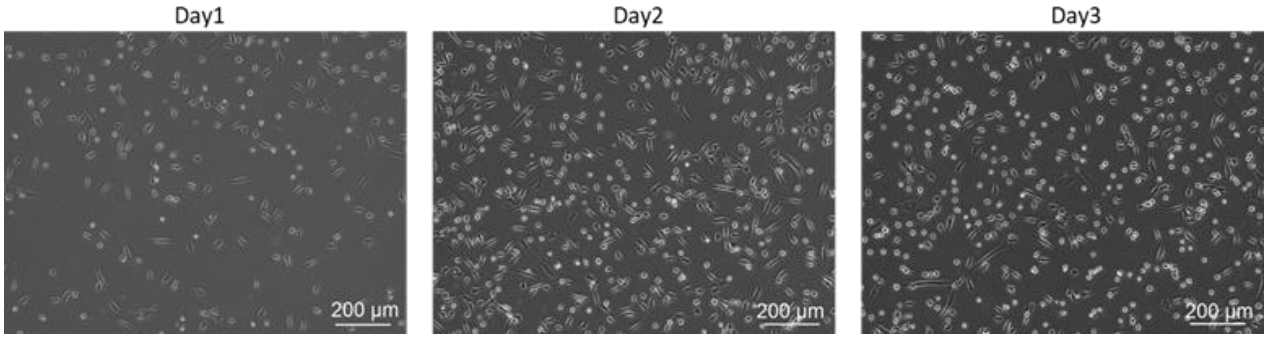

Figure 1. Mouse Skeletal Muscle Myoblasts (MSkMM) were recovered and seeded at 1X10^4/cm^2 in Mouse Skeletal Muscle Myoblast Growth Medium (Cat# MD-0064). Phase contrast images were taken at the indicated time post recovery.

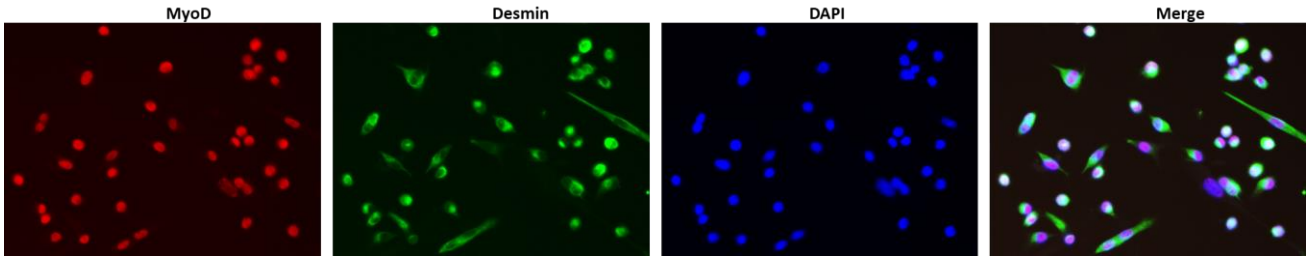

Figure 2. Mouse Skeletal Muscle Myoblasts (MSkMM) were fixed at DIV1 and ICC staining was performed using antibodies against MyoD and Desmin, separately.

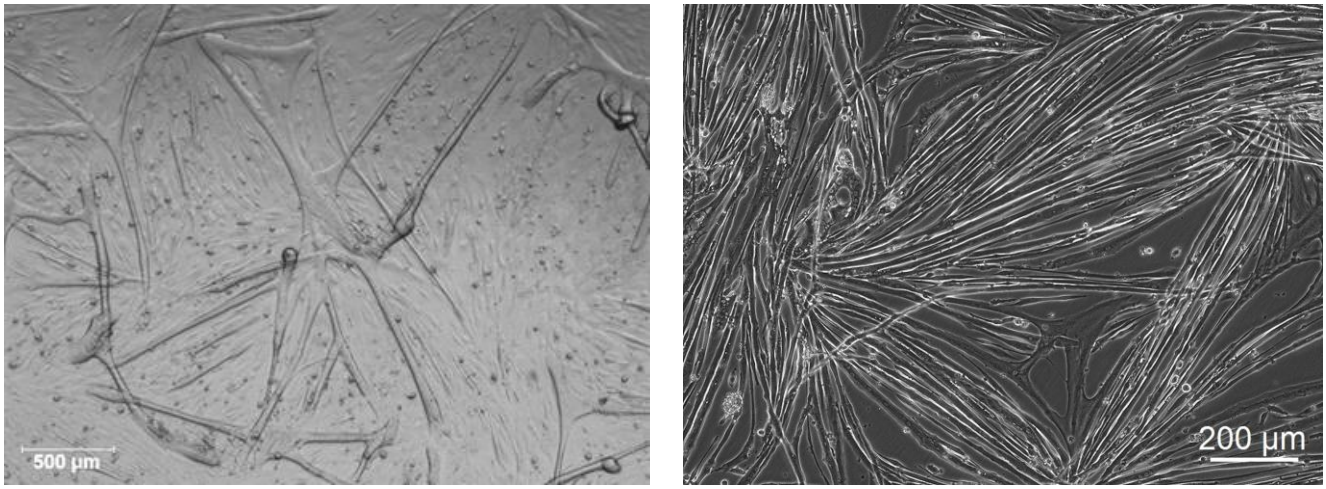

Figure 3. Mouse Skeletal Muscle Myoblasts (MSkMM) were cultured in Mouse Skeletal Muscle Myoblast Growth Medium (Cat# MD-0064) until the cells reach 70-80% confluency. The growth medium was replaced with Mouse Skeletal Muscle Myoblast Differentiation Medium (Cat# MD-0065) for 48 hours. The phase contrast image was shown indicating the formation of myotubes.

Product Details

Tissue	Gastrocnemius and tibialis anterior muscles of C57BL/6 mice
Package Size	0.5 x 10^6 cells/vial
Passage Number	P1
Shipped	Cryopreserved
Storage	Liquid nitrogen
Growth Properties	Adherent
Media	Mouse Skeletal Muscle Myoblast Growth Medium (Cat# MD-0064) Mouse Skeletal Muscle Myoblast Differentiation Medium (Cat# MD-0065)

Protocols

Coating plates/flasks

1. Thaw Matrigel (Fisher Scientific, Cat#CB-40234A) at 4ºC overnight.
2. Dilute Matrigel 1:500 in ice cold Ham's F-10 medium (ThermoFisher Scientific, Cat#12390035).
3. Add coating solution to flasks/plates (5 mL/T75 flask) and incubate plates at room temperature for 1 hour on a rocker.
4. Remove the matrgel and use plates immediately or store at 4ºC for up to 48 hours.

Thawing of Frozen Cells

1. Upon receipt of the frozen cells, it is recommended to thaw the cells and initiate the culture immediately in order to retain the highest cell viability.
2. To thaw the cells, put the vial in 37°C water bath with gentle agitation for 1-2 minutes. Keep the cap out of water to minimize the risk of contamination.
3. Pipette the cells into a 15 mL conical tube with 5 mL fresh **Mouse Skeletal Muscle Myoblast Growth Medium (Cat# MD-0064)**.
4. Centrifuge at 500 g for 5 minutes under room temperature.
5. Remove the supernatant and resuspend the cells in fresh Mouse Skeletal Muscle Myoblast Cells Growth Medium.
6. Culture the cell in the Matrigel pre-coated T75 flask. Change the medium daily until cells reach 60-70% confluence.

Safety Precaution: *it is highly recommended that protective gloves and clothing should be used when handling frozen vials.*

Standard Culture Procedure

1. Mouse Skeletal Muscle Myoblasts (MSkMM) can be cultured in **Mouse Skeletal Muscle Myoblast Growth Medium (MD-0064)**.
2. When cells reach ~60-70% confluence, remove the medium, and wash once with sterile PBS (5mL for one T75 flask).
3. Add 15 mL of Ca^{2+}/Mg^{2+}-free DPBS and incubate at 37ºC for 5-10 minutes.
4. Transfer the cells into a conical tube and rinse the plate with an additional 10 mL of DPBS.
5. Count cells and spin at 500 g for 5 minutes.
6. Seed the cells in the new pre-coated culture vessels at 1.5×10^4 cells/cm^2. Change the medium daily until cells reach 60-70% confluence.

Myotube Differentiation Protocol (24 well plate format)

1. Grow MSkMM on pre-coated 24-well plates and seed cells at 60,000 cells/cm^2 in **Mouse Skeletal Muscle Myoblast Growth Medium (Cat# MD-0064)**.
2. Once cells reach 70-80% confluency, replace the growth medium with **Mouse Skeletal Muscle Myoblast Differentiation Medium (Cat# MD-0065)**.
3. Myotubes can be readily visualized after 48-72 hours post differentiation (Figure 3).

References

1. R L Perry, M A Rudnick. Molecular mechanisms regulating myogenic determination and differentiation. Front Biosci. 2000, 5: D750-67
2. Roong Zhao, Alistair J Watt, Michele A Battle, Jixuan Li, Benjamin J Bondow, Stephen A Duncan. Loss of both GATA4 and GATA6 blocks cardiac myocyte differentiation and results in acardia in mice. Dev Biol. 2008, 317(2): 614–619.
3. Brendan Evano, Shahragim Tajbakhsh. Satellite cells and the muscle stem cell niche. Physiol Rev. 2013, 93(1):23-67.

Disclaimers