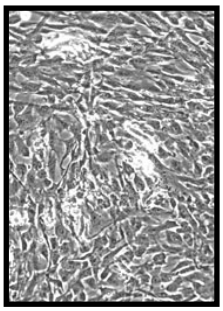
REF: P10977

SKELETAL MUSCLE INNOPROFILE™

HUMAN SKELETAL MUSCLE MYOBLASTS



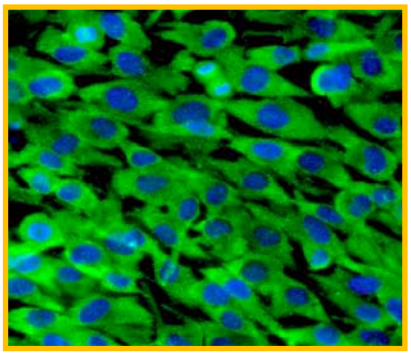
Product Type:	Cryo-preserved Skeletal Muscle Myoblasts
Catalog Number:	P10977
Source:	Human Muscle pectoral girdle
Number of Cells:	5 x 10^5 Cells / vial (1ml)
Storage:	Liquid Nitrogen

Human Skeletal Muscle Myoblasts (HSkMM) provided by Innoprot are isolated from human muscle of the pectoral girdle. HSkMM are cryopreserved on primary culture after purification and delivered frozen. HSkMM are guaranteed to further expand for 15 population doublings under the conditions provided in this technical sheet.

Skeletal muscle cells are one of the largest cells in the body. They are multinucleate formed by the fusion of myoblasts. Skeletal muscle regeneration is a complex process in which many factors are involved. When skeletal muscle suffers an injury, quiescent resident myoblasts called satellite cells are activated to proliferate, migrate, and finally differentia. The fusion of mononucleated cells to form multinucleated myotubes is a central event in skeletal muscle development. Controlling the onset and progression of this process is a complex set of interactions between myoblasts and their environment. Skeletal muscle cell culture is a useful model for studying this process of cell differentiation.

Recommended Medium

- Skeletal Muscle Cell Medium (Reference: P60124)



Product Characterization

Immunofluorescent method

- Myosin
- Actin
- Actinin

The cells test negative for HIV-1, HBV, HCV, mycoplasma, bacteria, yeast and fungi

Product Use

THESE PRODUCTS ARE FOR RESEARCH USE ONLY. Not approved for human or veterinary use, for application to humans or animals, or for use in vitro diagnostic or clinical procedures

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INSTRUCTIONS FOR CULTURING CELLS

IMPORTANT: Cryopreserved cells are very delicate. Thaw the vial in a 37 °C waterbath and return them to culture as quickly as possible with minimal handling!

Set up culture after receiving the order:

1. Prepare a poly-L-lysine coated flask (2 µg/cm^2, T-75 flask is recommended). Add 10 ml of sterile water to a T-75 flask and then add 15 µl of poly-L-lysine stock solution (10 mg/ml). Leave the flask in incubator overnight (minimum one hour at 37°C incubator).

2. Prepare complete medium: decontaminate the external surfaces of medium and medium supplements with 70% ethanol and transfer them to sterile field. Aseptically open each supplement tube and add them to the basal medium with a pipette. Rinse each tube with medium to recover the entire volume.

3. Rinse the poly-L-lysine coated flask with sterile water twice and add 20 ml of complete medium to the flask. Leave the flask in the hood and go to thaw the cells.

4. Place the vial in a 37°C waterbath, hold and rotate the vial gently until the contents are completely thawed. Remove the vial from the waterbath immediately, wipe it dry, rinse the vial with 70% ethanol and transfer it to a sterile field. Remove the cap, being careful not to touch the interior threads with fingers. Using a 1 ml eppendorf pipette gently resuspend the contents of the vial.

5. Dispense the contents of the vial into the equilibrated, poly-L-lysine coated culture vessels. A seeding density of 5,000 cells/cm2 is recommended.

Note: Dilution and centrifugation of cells after thawing are not recommended since these actions are more harmful to the cells than the effect of DMSO residue in the culture. It is also important that HNPC are plated in poly-L-lysine coated flask that promotes cell attachment and growth.

6. Replace the cap or cover, and gently rock the vessel to distribute the cells evenly. Loosen cap if necessary to permit gas exchange.

7. Return the culture vessels to the incubator.

8. For best result, do not disturb the culture for at least 16 hours after the culture has been initiated. Change the growth medium the next day to remove the residual DMSO and unattached cells, then every other day thereafter.

Maintenance of Culture:

1. Change the medium to fresh supplemented medium the next morning after establishing a culture from cryopreserved cells.

2. Change the medium every three days thereafter, until the culture is approximately 70% confluent.

3. Once the culture reaches 70% confluence, change medium every other day until the culture is approximately 90% confluent.

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Subculture:

1. Subculture when the culture reaches 90% confluency or above.

2. Prepare poly-L-lysine-coated culture vessels (2 µg/cm^2) one day before subculture.

3. Warm complete medium, trypsin/EDTA solution (T/E), Trypsin neutralization solution (TNS), and DPBS (Ca^{++} - and Mg^{++} -free) to room temperature. We do not recommend warming reagents and medium in a 37°C water bath prior to use.

Note: *DPBS, trypsin/EDTA solution & trypsin neutralization solution are included in the "Primary Cells Detach Kit provided by Innoprot (Cat. Nº P60305).*

4. Rinse the cells with DPBS.

5. Add 8 ml of DPBS and then 2 ml of T/E solution into flask (in the case of a T-75 flask). Gently rock the flask to ensure complete coverage of cells by T/E solution. Incubate the flask in a 37°C incubator for 1 to 2 minutes or until cells completely round up. Use a microscope to monitor the change in cell morphology.

6. During incubation, prepare a 50 ml conical centrifuge tube with 5 ml of fetal bovine serum (FBS).

7. Transfer T/E solution from the flask to the 50 ml centrifuge tube (a small percent of cells may detach) and continue to incubate the flask at 37°C for another 1 to 2 minutes (no solution in the flask at this moment).

8. At the end of incubation, gently tap the side of the flask to dislodge cells from the surface. Check under a microscope to make sure that all cells detach.

9. Add 5 ml of TNS solution to the flask and transfer detached cells to the 50 ml centrifuge tube. Rinse the flask with another 5 ml of TNS to collect the residual cells.

10. Examine the flask under a microscope for a successful cell harvest by looking at the number of cells being left behind; there should be less than 5%.

11. Centrifuge the 50 ml centrifuge tube at 1000 rpm for 5 minutes. Resuspend cells in culture medium.

12. Count and plate cells in a new poly-L-lysine-coated culture vessel with the recommended cell density.

Caution: *Handling human derived products is potentially bioharzadous. Although each cell strain testes negative for HIV, HBV and HCV DNA, diagnostic tests are not necessarily 100% accurate, therefore, proper precautions mush be taken to avoid inadvertent exposure. Always wear gloves and safety glasses when working these materials. Never mouth pipette. We recommend following the universal procedures for handling products of human origin as the minimum precaution against contamination [1].*

[1]. Grizzle, W. E., and Polt, S. S. (1988) Guidelines to avoid personal contamination by infective agents in research laboratories that use human tissues. *J Tissue Culture Methods.* 11(4).

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