Mouse Schwann Cells (MSC)

Catalog #M1700-57

Cell Specification

Schwann cells are neural crest derivatives that ensheathe and myelinate axons of peripheral nerves [1]. Each Schwann cell wraps around the shaft of an individual peripheral axon, forming myelin sheaths along segments of the axon. Schwann cells play important roles in the development, function, and regeneration of peripheral nerves. When an axon is dying, the Schwann cells surrounding it aid in its digestion, leaving an empty channel formed by successive Schwann cells, through which a new axon may then grow from a severed end. The number of Schwann cells in peripheral nerves is tightly regulated [2]. Their proliferation *in vitro* can be stimulated by various growth factors including PDGF, FGF, neuregulin, and others [3]. Schwann cells provide a relatively simple, well-defined, and accessible mammalian model for the study of a number of developmental questions. It is also of particular clinical importance to understand the biology of Schwann cells, not only in the context of neuropathies and nerve regeneration, but also because the cells or their precursors may be especially well suited for implants to facilitate repair in the CNS.

MSC from ScienCell Research Laboratories are isolated from postnatal day 8 C57BL/6 mouse sciatic nerve. MSC are cryopreserved as primary cultures and delivered frozen. Each vial contains >5 x 10^5 cells in 1 ml volume. MSC are characterized by immunofluorescence with antibodies specific to S100, GFAP, and CD90. MSC are negative for mycoplasma, bacteria, yeast, and fungi. MSC are guaranteed to further expand for 5 population doublings under the conditions provided by ScienCell Research Laboratories.

Recommended Medium

It is recommended to use Schwann Cell Medium (SCM, Cat. #1701) for culturing MSC *in vitro*.

Product Use

MSC are for research use only. They are not approved for human or animal use, or for application in *in vitro* diagnostic procedures.

Storage

Upon receiving, directly and immediately transfer the cells from dry ice to liquid nitrogen and keep the cells in liquid nitrogen until they are needed for experiments.

Shipping

Dry ice.

References

[1] Jessen KR, Mirsky R. (1999) "Schwann cells and their precursors emerge as major regulators of nerve development." *Trends Neurosci*. 22: 402-10.

[2] Syroid DE, Maycox PR, Burrola PG, Liu N, Wen D, Lee KF, Lemke G, Kilpatrick TJ. (1996) "Cell death in the Schwann cell lineage and its regulation by neuregulin." *Proc Natl Acad Sci USA*. 93: 9229-34.

[3] Rahmatullah M, Schroering A, Rothblum K, Stahl RC, Urban B, Carey DJ. (1998) "Synergistic regulation of Schwann cells proliferation by heregulin and forskolin." *Mol Cell Biol*. 18: 6245-52.

Instructions for culturing cells

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

Initiating the culture:

1. Prepare a poly-L-lysine-coated culture vessel (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, Cat. #0413). Leave the vessel in a 37°C incubator overnight (minimum one hour).
2. Prepare complete medium. Decontaminate the external surfaces of medium bottle and medium supplement tubes with 70% ethanol and transfer them to a sterile field. Aseptically transfer supplement to the basal medium with a pipette. Rinse the tube with medium to recover the entire volume.
3. Rinse the poly-L-lysine-coated vessel with sterile water twice and then add 15 ml of complete medium. Leave the vessel in the sterile field and proceed to thaw the cryopreserved cells.
4. Place the frozen vial in a 37°C water bath. Hold and rotate the vial gently until the contents completely thaw. Remove the vial from the water bath promptly, wipe it down with 70% ethanol and transfer it to the sterile field.
5. Remove the cap carefully without touching the interior threads. Gently resuspend and dispense the contents of the vial into the equilibrated, poly-L-lysine-coated culture vessel. A seeding density of ≥10,000 cells/cm^2 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 residual DMSO in the culture. It is also important that cells are plated in poly-L-lysine-coated culture vessels to promote cell attachment.
6. Replace the cap or lid and gently rock the vessel to distribute the cells evenly. Loosen cap if necessary to allow gas exchange.
7. Return the culture vessel to the incubator.
8. For best results, do not disturb the culture for at least 16 hours after the culture has been initiated. Refresh culture medium the next day to remove residual DMSO and unattached cells, then every other day thereafter.

Maintaining the culture:

1. Refresh supplemented culture 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% confluency, change medium every other day until the culture is approximately 90% confluent.

Subculturing:

1. Subculture when the culture reaches 90-95% confluency.
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, Cat. #0103), T/E neutralization solution (TNS, Cat. #0113), and DPBS (Ca^{++} and Mg^{++} free, Cat. #0303) to **room temperature**. We do not recommend warming reagents and medium in a 37ºC water bath prior to use.
4. Rinse the cells with DPBS.
5. Add 9 ml of DPBS and then 1 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 37ºC incubator for 1 minute or until cells start to 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, Cat. #0500).
7. Transfer T/E solution from the flask to the 50 ml centrifuge tube (a few percent of cells may detach) and continue to incubate the flask at 37ºC for another minute (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 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 residual cells.
10. Examine the flask under microscope for a successful cell harvest by looking at the number of cells left behind; there should be less than 5%.

    Note: Use ScienCell T/E solution that is optimized to minimize cell damages due to over trypsinization.
11. Centrifuge the 50 ml centrifuge tube at 1000 rpm for 5 min. 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 animal-derived products is potentially biohazardous. Always wear gloves and safety glasses when working with 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 WE, Polt S. (1988) "Guidelines to avoid personal contamination by infective agents in research laboratories that use human tissues." *J Tissue Cult Methods*. 11: 191-9.