



## Mouse Schwann Cells (MSC)

Catalog Number: M1700-57

### Cell Specification

Schwann cells are neural crest derivatives that ensheath and myelinate axons of peripheral nerves [1]. They wrap individually around the shaft of peripheral axons, forming myelin sheath 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. This leaves an empty channel formed by successive Schwann cells, through which a new axon may grow from a severed end. The number of Schwann cells in peripheral nerve is tightly regulated [2]. Their proliferation *in vitro* can be stimulated by growth factors including PDGF, FGF, neuregulin and others [3]. The 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 precursor might be especially well suited as implants to facilitate repair in the CNS.

MSC from ScienCell Research Laboratories are isolated from neonate day two C57BL/6 mouse sciatic nerves. MSC are cryopreserved either at primary or passage one culture and delivered frozen. Each vial contains  $>5 \times 10^5$  cells in 1 ml volume. MSC are characterized by immunofluorescent method with antibodies to S-100, GFAP and CD90. MSC are negative for mycoplasma, bacteria, yeast and fungi. MSC are guaranteed to further expand 5 population doublings in the conditions provided by ScienCell Research Laboratories.

### Recommended Medium

It is recommended to use Schwann Cell Medium (SCM, Cat. No. 1701) for the culturing of 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

Transfer cells directly and immediately from dry ice to liquid nitrogen upon receiving and keep the cells in liquid nitrogen until cell culture is needed for experiments.

### Shipping

Dry ice.

### Reference

- [1] Jessen, K. R. and Mirsky, R. (1999) Schwann cells and their precursors emerge as major regulators of nerve development. *Trends Neurosci.* 22:402-410.
- [2] Syroid, D. E., Maycox, P. R., Burrola, P. G., Liu, N., Wen, D., Lee, K. F., Lemke, G., Kilpatrick, T. J. (1996) Cell death in the Schwann cell lineage and its regulation by neuregulin. *Proc. Natl. Acad. Sci. USA* 93:9229-9234.
- [3] Rahmatullah, M., Schroering, A., Rothblum, K., Stahl, R. C., Urban, B and Carey, D. J. (1998) Synergistic regulation of Schwann cells proliferation by heregulin and forskolin. *Mol. Cell. Biol.* 18:6245-6252.

## Instruction for culturing cells

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Caution: 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  $\mu\text{g}/\text{cm}^2$ , T-75 flask is recommended). Add 10 ml of sterile water to a T-75 flask and then add 15  $\mu\text{l}$  of poly-L-lysine stock solution (10 mg/ml, ScienCell cat. no. 0413). 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, and transfer it to a sterile field. Rinse the vial with 70% ethanol, and then wipe to remove excess. Remove the cap, being careful not to touch the interior threads with fingers. Using 1 ml eppendorf pipet gently resuspends 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  $\geq 10,000$  cells/ $\text{cm}^2$  is recommended.
6. *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 cells are plated in poly-L-lysine coated culture vessels that promote cell attachment.*
7. Replace the cap or cover, and gently rock the vessel to distribute the cells evenly. Loosen caps if necessary to permit gas exchange.
8. Return the culture vessels to the incubator.
9. 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. A health culture will display typical oval-shaped cell body, with a prominent nucleus and bipolar extensions, giving an overall spindle shape; and the cell number will be doubled after two to three days in culture.

### **Maintenance of Culture:**

1. Change the medium to fresh supplemented medium the next morning after establishing a culture from cryopreserved cells. For subsequent subcultures, change medium 48 hours after establishing the subculture.
2. Change the medium every other day thereafter, until the culture is approximately 50% confluent.
3. Once the culture reaches 50% confluence, change medium every day until the culture is approximately 80% confluent.

### **Subculture:**

1. Subculture the cells when they are over 90% confluent.
2. Prepare poly-L-lysine coated cell culture flasks ( $2 \mu\text{g}/\text{cm}^2$ ).
3. Warm medium, trypsin/EDTA solution (T/E, cat. no. 0103), trypsin neutralization solution (TNS, cat. no. 0113), and DPBS ( $\text{Ca}^{++}$  and  $\text{Mg}^{++}$  free, cat. no. 0303) to **room temperature**. We do not recommend warming the reagents and medium at  $37^\circ\text{C}$  waterbath prior to use.
4. Rinse the cells with DPBS.
5. Add 8 ml of DPBS first and then 2 ml of trypsin/EDTA solution into flask (in the case of T-75 flask); gently rock the flask to make sure cells are covered by trypsin/EDTA solution; incubate the flask at  $37^\circ\text{C}$  incubator for 2 minutes or until cells are completely rounded up (monitored with inverted microscope). During incubation, prepare a 50 ml conical centrifuge tube with 5 ml of fetal bovine serum (FBS, cat. no. 0500); transfer trypsin/EDTA solution from the flask to the 50 ml centrifuge tube (a few percent of cells may detached); continue incubate the flask at  $37^\circ\text{C}$  for 1 or 2 minutes more (no solution in the flask at this moment); at the end of trypsinization, one hand hold one side of flask and the other hand gently tap the other side of the flask to detach cells from attachment; check the flask under inverted microscope to make sure all cells are detached, add 5 ml of trypsin neutralization solution to the flask and transfer detached cells to the 50 ml centrifuge tube; add another 5 ml of TNS to harvest the residue cells and transfer it to the 50 ml centrifuge tube. Examine the flask under inverted microscope to make sure the cell harvesting is successful by looking at the number of cells left behind. There should be less than 5%.

*Note: Use ScienCell Research Laboratories' trypsin/EDTA solution that is optimized to minimize the killing of the cells by over trypsinization.*

6. Centrifuge the 50 ml centrifuge tube (harvested cell suspension) at 1000 rpm (*Beckman Coulter Allegra 6R centrifuge or similar*) for 5 min; re-suspend cells in growth medium.

7. Count cells and plate cells in a new, poly-L-lysine coated flask with cell density as recommended.

*Caution: Handling animal derived products is potentially biohazardous. Always wear gloves and safety glasses when working these materials. Never mouth pipette. We recommend following the universal procedures for handling products of animal 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).