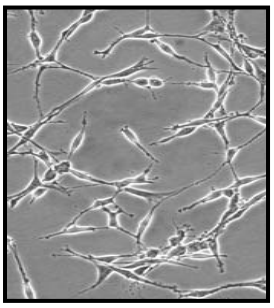
REF: P10301

NEUROSCIENCES INNOPROFILE™
RAT SCHWANN CELLS



Product Type:	Cryo-preserved Schwann Cells
Catalog Number:	P10301
Source:	Rat Sciatic Nerves
Number of Cells:	5 x 10^5 Cells / vial (1ml)
Storage:	Liquid Nitrogen

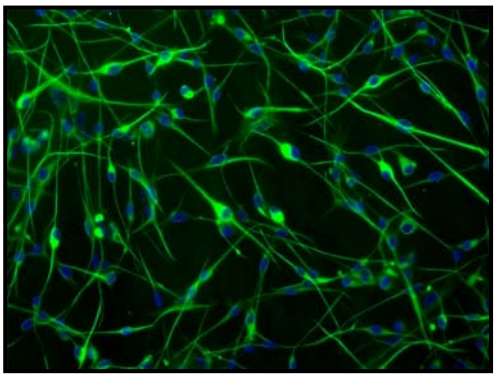
Rat Schwann Cells (RSC) provided by Innoprot are isolated from rat sciatic nerves. RSC are cryopreserved at passage one and delivered frozen. RSC are guaranteed to further expand for 5 population doublings in the conditions provided by Innoprot.

Schwann cells are neural crest derivatives that ensheathe and myelinate axons of peripheral nerves. They wrap individually around the shaft of peripheral axons, forming a layer or myelin sheath along segments of the axon. Schwann cells play important roles in the development, function and regeneration of peripheral nerves. Their proliferation in vitro can be stimulated by polypeptide growth factors including PDGF, FGF, neuregulin and others. The Schwann cells provide a well-defined 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.

Recommended Medium

- Schwann Cell Medium

  (Reference: P60123)



Product Characterization

Immunofluorescent method

- S-100
- GFAP
- CD90

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.

INNOVATIVE TECHNOLOGIES IN BIOLOGICAL SYSTEMS, S.L.
Parque Tecnológico Bizkaia, Edf. 502, 1ª Planta | 48160 | Derio | Bizkaia
Tel.: +34 944005355 | Fax: +34 946579925
innoprot@innoprot.com | www.innoprot.com

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 150 µl of poly-L-lysine stock solution (1 mg/ml, Innoprot # PLL). 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 re-suspend 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/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 DMSO residue in the culture.

It is also important that cells 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.

INNOVATIVE TECHNOLOGIES IN BIOLOGICAL SYSTEMS, S.L.
Parque Tecnológico Bizkaia, Edf. 502, 1ª Planta | 48160 | Derio | Bizkaia
Tel.: +34 944005355 | Fax: +34 946579925
innoprot@innoprot.com | www.innoprot.com

Subculture:

1. Subculture the cells when they are over 90% confluent.

2. Prepare poly-L-lysine coated cell culture flasks (2 µg/cm²).

3. Warm medium, trypsin/EDTA solution (T/E Solution), trypsin neutralization solution (TNS), and DPBS (Ca ++ and Mg++ free) to room temperature. We do not recommend warming the reagents and medium at 37°C waterbath prior to use.

Note: DPBS, trypsin/EDTA solution & trypsin neutralization solution are included in the "Primary Cells Detach Kit provided by Innoprot (Cat. N° P60305).

4. Rinse the cells with DPBS.

5. Add 10 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 37°C 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.

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 37°C 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%.

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 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).

INNOVATIVE TECHNOLOGIES IN BIOLOGICAL SYSTEMS, S.L.
Parque Tecnológico Bizkaia, Edf. 502, 1ª Planta | 48160 | Derio | Bizkaia
Tel.: +34 944005355 | Fax: +34 946579925
innoprot@innoprot.com | www.innoprot.com