NEUROMICS

Human Primary Schwann Cells

Catalog #: HMP303
Passage Number: 1
Storage: Liquid Nitrogen

Cell #: 5X10^5 cells
Product Format: Cryopreserved
Growth Properties: Adherent

General Information

Schwann cells are neural crest derivatives that ensheathe and myelinate axons of peripheral nerves. 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. Their proliferation in vitro can be stimulated by various growth factors including PDGF, FGF, neuregulin, and others. 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.

Human Schwann Cells (HSwC) are isolated from human spinal nerve. HSwC are cryopreserved at passage one and delivered frozen. Each vial contains >5 x 10^5 cells in 1 ml volume. HSwC are characterized by immunofluorescence with antibodies specific to S100, GFAP, and CD90. HSwC can expand for 10 population doublings in our Schwann Growth medium (cat # SGM001).

Characterization of the cells

HSwC are characterized by immunofluorescence with antibodies specific to S100, GFAP, and CD90
HBMECs are negative for HIV-1, HBV, HCV, mycoplasma, bacteria, yeast, and fungi

Recommended Products

- Schwann Growth Medium – SGM001
  - Schwann Growth Medium

- AlphaBioCoat Solution – AC001
  - Premium Smooth Coat Solution. Biocompatible complex of extracellular matrix binding solution with growth factors. Ideal for culturing cells from frozen.

Shipping

Shipped on dry ice frozen in a vial.

Handling of Arriving Cells

Store in liquid nitrogen to keep the cells frozen or thaw cells according to the protocol for culture.

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

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Live/proliferating cells:

1. Upon arrival, incubate the flask containing cells and media in an incubator (37°C, 5% CO2) for 3-5 hours to recover from transportation.
2. Carefully place the vessel in a biosafety cabinet and spray the outer side of flask with 70% ethanol to disinfect.
3. Let it air dry. Carefully open the vessel while keeping it in upright standing position.
4. If the cells you received are suspension carefully transfer cell suspension is ready to be plated in the desired culture flask containing appropriate growth media. Incubate again the culture flask in incubator (37°C, 5% CO2). Change media or subculture as needed.
5. If the cells you received are adherent: Carefully aspirate the media and add fresh appropriate growth media to flask and let it incubate overnight at 37°C, 5% CO2). Change media or subculture as needed.

Frozen Cells Protocol:

1. Remove the cryovial from the liquid nitrogen storage tank.
2. Thaw the cells quickly by placing the lower half of the vial into a 37°C water bath while agitating gently, remove after 60 seconds. Keep the cap out of the water to avoid contamination. There should still be a few ice crystals left after thawing (it is important not to over-thaw the cryovials as the presence of DMSO is toxic to the cells).
3. Decontaminate the vial by spraying and wiping the exterior of the vial with 70% ethanol. From this point onwards, all operations should be strictly carried out inside a laminar flow hood in aseptic conditions.
4. Gently re-suspend the cells in the vial and transfer the cell suspension into a 15 mL sterile conical tube containing 5 mL of pre-warmed, complete media using a sterile transfer pipette.
5. Centrifuge the cells at 1500 rpm for approximately 3 minutes to pellet (actual centrifuge duration may vary)
6. Check the clarity of the supernatant to ensure that all cells are pulled down into the pellet. Aspirate out the supernatant without disturbing the cell pellet.
7. Re-suspend the cell pellet in fresh, pre-warmed culture media and transfer the cells into a culture vessel. Gently rock the culture vessel to distribute the cells evenly.
8. Incubate the culture at 37°C, 5% CO2. Incubate for at least 24 hours before processing the cells for downstream experiments.

Sub Culture Protocol:

Coating T-25 flasks: Add 2ml Smooth Coat Solution (SC300) into one T25 flask. Make sure whole surface of the flask is covered with the Smooth coat solution, transfer into incubator. 30 minutes later, dispose Smooth Coat Solution by aspiration, gently rinse flask with PBS. The flask is ready to be used (no need for overnight incubation when coated with Smooth Coat Solution).

1. Rinse the cells in T25 flask with 5ml PBS (Room Temperature, RT) twice.
2. Add 2ml of Trypsin/EDTA (RT) into T25 flask (make sure the whole surface of the T25 flask is covered with Trypsin/EDTA), and gently dispose the Trpsin/EDTA solution within 10 seconds with aspiration. Leave the T25 flask with the cells at RT for 1 minute (the cells will normally come off the surface within 1 minute).
3. Suspend the cells with 20ml of Schwann Growth medium and the cell suspension is transferred directly into 2 x pre-coated T25 flasks (5ml each, and the cells are subculture at 1:2 ratio)
    A) Culture medium is changed every 2 days. The cells normally become confluent within 7 days (when split at a 1:2 ratio).

Freezing Protocol:

1. Always freeze down cells at a high concentration and at as low a passage number as possible. Ensure that the cells are at least 90% viable before freezing. Always use proper aseptic technique and work in a laminar flow hood. Always wear personal protective equipment when working with liquid nitrogen.
2. Harvest log phase cells (with > 90% viability): For adherent cells, gently detach the cells from the culture vessel to collect cells into a centrifuge tube following the Subculturing Protocol (step 1 to step 6). For suspension cells, harvest all cells into a centrifuge tube.
3. Determine viable cell density and calculate the required volume of Cryopreservation Medium needed. We recommend freezing cells at 1.0 to 2.5 x 106 cells/ml.
4. Centrifuge the cell suspension at 1500 rpm for 3 minutes.
5. Aseptically, aspirate out the supernatant without disturbing the pellet.
6. Re-suspend the cell pellet in Cryopreservation Medium at the appropriate cell density.
7. Dispense the cell suspension into cryovials and freeze according to your laboratory standard (i.e. controlled rate freezing at approximately 1 oC decrease per minute).
8. Transfer the frozen cells into liquid nitrogen storage (in the gas phase above the liquid nitrogen) for long-term storage.

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