Schwann cell isolation

Preparation and MACS® Separation of Schwann cells from rat peripheral nerve tissue biopsies

Contents

1. Description

1.1 Principle of Schwann cell isolation

Schwann cells can be isolated from rat peripheral nerve tissue biopsies through a series of enzymatic, trituration and cultivation steps, concluding with a MACS® MicroBeads selection of Schwann cells based on their surface expression of p75 (or NGFR, nerve growth factor receptor).

Initially, the epineurium (the outermost layer of connective tissue surrounding a peripheral nerve) can be removed by simply pulling out the nerve fascicles – the bundles of individual nerve fibers. Following enzymatic degradation of the extracellular matrix, mechanical trituration further breaks up the tissue structure to facilitate *in vitro* cultivation of the cells as a monolayer in culture flasks coated with poly-L-ornithine and laminin, which aid the adhesion of peripheral nerve cells to plastic surfaces.

However, cell extracts from peripheral nerve tissue biopsies are very heterogeneous, containing a high number of fibroblast cells in addition to Schwann cells. Therefore, a positive selection of Schwann cells using the p75 antigen allows for the specific and high-purity enrichment of Schwann cells using magnetic cell separation.[1] Schwann cells are then immediately ready for further cultivation or *in vitro* analysis.

1.2 Reagent and instrument requirements

Reagent and instrument requirements for biopsies preparation

- Culture medium: DMEM/F-12 (Invitrogen # 12634-010), supplemented with 10% fetal calf serum, 0.365 g/mL L-glutamine (Invitrogen # 25030-081) and Penicillin-Streptomycin (Invitrogen # 15070-063).
- Enzyme mixture: 250 U/mL Hyaluronidase Type I-S (Sigma # H3506) and 160 U/mL Collagenase Type I (Sigma # C1639) in culture medium.
- Trypsin-EDTA (0.05%/0.53 mM, Invitrogen # 25300-054).
- 10 cm^2 sterile petri dish.
- Sterile forceps.
- Sterile scalpel.
- 10 mL glass pipette.
- 10 mL syringe with 20-, 22-, and 27-gauge needles.
- 75 cm^2 culture flasks (e.g. BD Biosciences # 353136) coated with poly-L-ornithine and laminin.
- Centrifuge.

Reagent and instrument requirements for MACS® Separation of Schwann cells

- Separation buffer: Prepare a solution containing phosphate-buffered saline (PBS), pH 7.2, 0.5% bovine serum albumin (BSA), and 2 mM EDTA by diluting MACS BSA Stock Solution (# 130-091-376) 1:20 with autoMACS™ Rinsing Solution (# 130-091-222). Keep buffer cold (2–8 °C). Degas buffer before use, as air bubbles could block the column.
- Wash buffer: Prepare a solution containing PBS, pH 7.2, and 2 mM EDTA. Keep buffer cold (2–8 °C).
- PBS, pH 7.2.
- Anti-p75 primary antibody, clone 192-IgG (Chemicon # MAB365).
- Rat Anti-Mouse IgG1 MicroBeads (# 130-047-102).
- MS Column (# 130-042-201).
- 15 mL conical tubes.
- 50 mL conical tubes.
- Hemocytometer.
- (Optional) Propidium iodide (PI) or 7-AAD for flow cytometric exclusion of dead cells.

2. Protocol

2.1 Schwann cell preparation

▲ For cell culture experiments subsequent to tissue dissociation, all steps should be performed under sterile conditions.

1. Wash biopsy in 25 mL of culture medium and place it in a sterile petri dish.
2. Carefully remove the epineurium from the nerve by pulling out the individual nerve fascicles using forceps.
3. Wash the nerve fascicles in 25 mL of culture medium, then subdivide into 1 millimeter–long pieces.
4. Resuspend the tissue pieces in enzyme mixture and incubate for 18 hours at 37 °C.

   ▲ **Note:** Use 10 mL of enzyme mixture for a maximum of 500 mg peripheral nerve tissue per petri dish.
5. After incubation, use a 10 mL glass pipette to gently triturate the tissue pieces.

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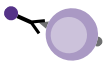
Miltenyi Biotec GmbH
Friedrich-Ebert-Str. 68
51429 Bergisch Gladbach, Germany
Phone +49 2204 8306-0 Fax +49 2204 85197

Miltenyi Biotec Inc.
12740 Earhart Avenue, Auburn CA 95602, USA
Phone 800 FOR MACS, +1 530 888 8871
Fax +1 530 888 8925

6. Using a 10 mL syringe with a 20-gauge needle attached, continue triturating until pieces can be readily drawn into the syringe. Repeat the trituration using a 22-gauge and then 27-gauge needle.
7. Centrifuge cells at 300×g for 5 minutes.
8. Aspirate the supernatant and resuspend cells in 10 mL of culture medium. Triturate gently once more with the 27-gauge needle.
9. Wash cells twice in 10 mL culture medium, centrifuging at 300×g for 5 minutes after each wash.
10. Plate cells at a sub-confluent concentration in culture medium on poly-L-ornithine/laminin-coated 75 cm^2 culture flasks. Cultivate cells at 37 °C, 5% CO_2 and >95% humidity for 24 hours before starting the magnetic labeling of Schwann cells.

    ▲ **Note:** Use approximately three 75 cm^2 culture flasks per 500 mg peripheral nerve tissue.



2.2 Magnetic labeling of Schwann cells

▲ Work fast, keep cells cold, and use pre-cooled solutions. This will prevent capping of antibodies on the cell surface and non-specific cell labeling.

▲ Volumes for magnetic labeling given below are for up to 10^7 total cells. When working with fewer than 10^7 cells, use the same volumes as indicated. When working with higher cell numbers, scale up all reagent volumes and total volumes accordingly (e.g. for $2×10^7$ total cells, use twice the volume of all indicated reagent volumes and total volumes).

▲ When working with MicroBeads the recommended incubation temperature is 2–8 °C. Working on ice may require increased incubation times. Higher temperatures and/or longer incubation times may lead to non-specific cell labeling.

1. Wash cells in the culture flask with PBS.
2. Add 1 mL of pre-warmed (37 °C) Trypsin/EDTA to each culture flask and incubate at 37 °C until cells are detached.

   ▲ **Note:** Time of trypsinization may vary but usually cells dissociate within 5 to 15 minutes.
3. Collect the cells in 10 mL of culture medium and transfer cells to a single 15 mL conical tube by pooling each culture flask.
4. Centrifuge cell suspension at 300×g for 10 minutes. Aspirate supernatant completely.
5. Determine the number of cells using a hemocytometer and transfer cells into a 15 mL conical tube.
6. Wash cells by adding 10 mL of wash buffer and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.
7. Resuspend cell pellet in 100 µL of separation buffer containing 5 µL anti-p75 primary antibody (clone 192-IgG).
8. Mix well and incubate for 10 minutes at room temperature.
9. Wash cells by adding 10 mL of wash buffer and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.
10. Resuspend cell pellet in 80 µL of separation buffer per 10^7 total cells.
11. Add 20 µL of Rat Anti-Mouse IgG1 MicroBeads per 10^7 total cells.
12. Mix well and incubate for 15 minutes in the refrigerator (2–8 °C).
13. Wash cells by adding 10 mL of wash buffer and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.
14. Resuspend up to 10^8 cells in 500 µL of separation buffer.

    ▲ **Note:** For higher cell numbers, scale up buffer volume accordingly.

    ▲ **Note:** For depletion with LD Columns, resuspend up to $1.25×10^8$ cells in 500 µL of buffer.
15. Proceed to magnetic separation (2.3).

2.3 Magnetic separation

Magnetic separation with an MS Column

1. Place column in the magnetic field of a suitable MACS Separator. For details see respective MACS Column data sheet.
2. Prepare MS Column by rinsing with 500 µL of separation buffer.
3. Apply cell suspension onto the column.
4. Collect unlabeled cells that pass through and wash column with 500 µL of separation buffer. Collect total effluent; this is the unlabeled cell fraction. Perform washing steps by adding buffer three times. Only add new buffer when the column reservoir is empty.
5. Remove column from the separator and place it on a suitable collection tube.
6. Pipette 1 mL of separation buffer onto the column. Immediately flush out the magnetically labeled cells by firmly pushing the plunger into the column.

   ▲ **Note:** Keep handling times of cells in PBS/EDTA/BSA buffer to a minimum. Cells must only be stored in cell cuture medium after enrichment over the columns in order to preserve cell viability.
7. Centrifuge cells at 300×g for 5 minutes. Resuspend in 1 mL culture medium and count cells by using a hemocytometer.
8. Cells can either be taken directly into culture by re-plating on poly-L-ornithine/laminin-coated culture flasks or analyzed for purity by flow cytometry.

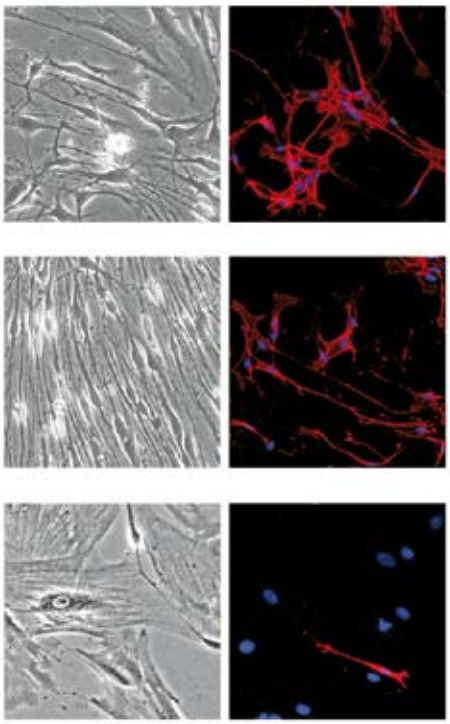
3. Example of a Schwann cell isolation

Schwann cells were prepared as described above, and analyzed by light microscopy (left panels) as well as immunofluorescent microscopy (right panels). For the immunofluorescent detection of Schwann cells, cells were stained using a fluorochrome-conjugated anti-p75 antibody (right panels, red) and nuclei counterstained using Hoechst 33342 (right panels, blue).

Unseparated cells

p75–positive fraction

p75–negative fraction



(Courtesy of Dr. Norbert Weidner, University of Regensburg, Germany.)

4. Reference

1. Vroemen, M. and Weidner, N. (2003) Purification of Schwann cells by selection of p75 low affinity nerve growth factor receptor expressing cells from adult peripheral nerve. J. Neurosci. Methods 124: 135–43.

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