Anti-O4 antibodies
human, mouse, rat

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1. Description

Components	1 mL monoclonal Anti-O4 antibodies, human, mouse, rat conjugated to various dyes.
	PE 130-095-887
	APC 130-095-891
	Biotin 130-095-895
Clone	O4 (isotype: mouse IgM).
Capacity	100 tests or up to 10^8 total cells.
Product format	Antibodies are supplied in buffer containing stabilizer and 0.05% sodium azide.
Storage	Store protected from light at 2–8 °C. Do not freeze. The expiration date is indicated on the vial label.

Cross-reactivity: The Anti-O4 antibody has been reported to react with chicken cells.

1.1 Background information

The O4 antigen, a sulfatide, which belongs to the class of glycosphingolipids, is a marker for oligodendrocytes. During oligodendrocyte development, O4 expression begins on late oligodendrocyte progenitors that are A2B5-positive. While A2B5 expression dispappears, O4 continues to be expressed. As the cells differentiate, they synthesize galactocerebroside, myelin basic protein (MBP), proteolipid protein (PLP), myelin oligodendrocyte glycoprotein (MOG), and O1. In the peripheral nervous system, O4 expression is found on Schwann cells.

1.2 Applications

- Identification and enumeration of $O4^+$ cells by flow cytometry.

1.3 Recommended antibody dilution

The recommended antibody dilution for all Anti-O4 conjugates is **1:11 for up to 10^6 cells/100 µL** of buffer for labeling of cells and analysis by flow cytometry. For Anti-O4 MicroBead-labeled cells use the same dilution.

1.4 Reagent requirements

- Buffer: Prepare a solution containing phosphate-buffered saline (PBS), pH 7.2, and 0.5% bovine serum albumin (BSA) by diluting MACS® BSA Stock Solution (# 130-091-376) 1:20 with PBS. Keep buffer cold (2–8 °C).

  ▲ **Note:** BSA can be replaces by other proteins such as appropriate serum albumin, appropriate serum, or fetal bovine serum (FBS).
- Neural Tissue Dissociation Kit (P) (# 130-092-628) or Neural Tissue Dissociation Kit (T) (# 130-093-231) for the generation of single-cell suspension from neural tissues.
- (Optional) gentleMACS™ Dissociator (# 130-093-235).
- FcR Blocking Reagent, mouse (# 130-092-575) or FcR Blocking Reagent, human (# 130-059-901) to avoid Fc receptor–mediated antibody labeling.
- (Optional) Anti-Biotin-VioBlue® (# 130-094-669), Anti-Biotin-FITC (# 130-090-857), Anti-Biotin-PE (# 130-090-756), or Anti-Biotin-APC (# 130-090-856) as secondary antibody reagent in combination with Anti-O4-Biotin.
- (Optional) Mouse IgM-PE (# 130-093-177), Mouse IgM-APC (# 130-093-176), or Mouse IgM-Biotin (# 130-093-175) for isotype control.
- (Optional) Propidium Iodide Solution (# 130-093-233) or 7-AAD for flow cytometric exclusion of dead cells without fixation.

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2. General protocol for immunofluorescent staining

▲ Volumes given below are for **up to 10^6** nucleated cells. When working with fewer than 10^6 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 2×10^6 nucleated cells, use twice the volume of all indicated reagent volumes and total volumes).

1. Determine cell number.
2. Centrifuge cell suspension at 300×g for 10 minutes. Aspirate supernatant completely.
3. Add 100 µL of buffer per 10^6 nucleated cells to the cell pellet.

   ▲ **Note:** If FcR Blocking Reagent, mouse is being used add 90 µL of buffer and 10 µL of the FcR Blocking Reagent, mouse directly before addition of the Anti-O4 antibody per 10^6 nucleated cells.

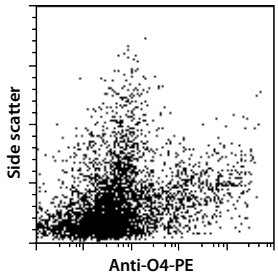
   ▲ **Note:** If FcR Blocking Reagent, human is being used add 80 µL of buffer and 20 µL of the FcR Blocking Reagent, human directly before addition of the Anti-O4 antibody per 10^6 nucleated cells.
4. Add 10 µL of the Anti-O4 antibody.
5. Mix well. Do not vortex. Incubate for 10 minutes in the dark in the refrigerator (2–8 °C).

   ▲ **Note:** Higher temperatures and/or longer incubation times may lead to non-specific cell labeling. Working on ice requires increased incubation times.
6. Wash cells by adding 1–2 mL of buffer and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.
7. (Optional) If Anti-O4-Biotin was used, resuspend the cell pellet in 100 µL of buffer, add 10 µL of anti-biotin antibody, and continue as described in steps 5 and 6.
8. Resuspend cell pellet in a suitable amount of buffer for analysis by flow cytometry or fluorescence microscopy.

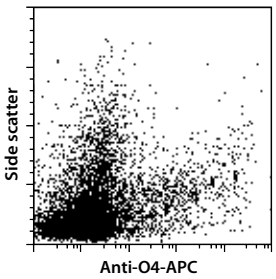
3. Examples of immunofluorescent staining with Anti-O4 antibodies

Mouse brain tissue postnatal day 6 was dissociated using the Neural Tissue Dissociation Kit (P) and the gentleMACS Dissociator.
Mouse brain cells were stained with Anti-O4 antibodies conjugated to PE (A) or APC (B) and analyzed by flow cytometry using the MACSQuant® Analyzer. Cells labeled with Anti-O4-Biotin (C) were stained with Anti-Biotin-APC (# 130-090-856). Cell debris and dead cells were excluded from the analysis based on scatter signals and propidium iodide fluorescence.

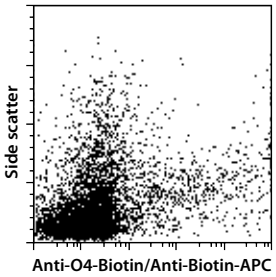
A)



B)



C)



4. References

1. Zhang, S.C. (2001) Defining glial cells during CNS development. Nat. Rev. Neurosci. 2: 840–843.
2. Sommer, I. and Schachner, M. (1981) Monoclonal antibodies (O1 to O4) to oligodendrocyte cell surfaces: an immunocytological study in the central nervous system. Dev. Biol. 83: 311–327.

All protocols and data sheets are available at **www.miltenyibiotec.com**.

Warnings

Reagents contain sodium azide. Under acidic conditions sodium azide yields hydrazoic acid, which is extremely toxic. Azide compounds should be diluted with running water before discarding. These precautions are recommended to avoid deposits in plumbing where explosive conditions may develop.

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