

Anti-Microfold (M) Cell antibodies

mouse

Contents

1. Description
 - 1.1 Background information
 - 1.2 Applications
 - 1.3 Recommended antibody dilution
 - 1.4 Reagent requirements
2. General protocol for immunofluorescent staining
3. Examples of immunofluorescent staining with Anti-Microfold (M) Cell antibodies
4. References

1. Description

Components	1 mL monoclonal Anti-Microfold (M) Cell antibodies, mouse conjugated to various dyes.
	PE 130-096-155
	APC 130-096-153
	pure 130-096-148
	or
	0.5 mL Anti-Microfold (M) Cell antibodies, mouse
	pure – functional grade 130-096-150
Clone	NKM 16-2-4 (isotype: rat IgG2c).
Capacity	100 tests or up to 10 ⁹ total cells. The unconjugated (pure) antibody is supplied at a concentration of 100 µg/mL. The functional grade antibody is supplied at a concentration of 1 mg/mL.
Product format	Antibodies are supplied in buffer containing stabilizer and 0.05% sodium azide. Functional grade antibodies are supplied in phosphate-buffered saline (PBS), pH 7.2. Endotoxin levels have been tested and do not exceed 0.01 ng/µg of protein. <i>The functional grade product contains no preservative and is sterile filtered; always handle under aseptic conditions.</i>
Storage	Store protected from light at 2–8 °C. Do not freeze. The expiration date is indicated on the vial label.

1.1 Background information

Clone NKM 16-2-4 specifically binds (1,2)-fucose-containing carbohydrate moieties on murine microfold cells (M cells or membranous cells). Villous M cells are located in follicle-associated epithelium of Peyer's patches (PPs) and nasopharynx-associated

lymphoid tissue (NALT). NKM 16-2-4 specifically binds to all subsets of M cells, but not epithelial cells or goblet cells located in PPs, NALT, and the intestinal villi in the gastrointestinal or respiratory tracts.

M cells have a high transcytotic activity and play an important role in the uptake of luminal antigens for induction of antigen-specific immune responses. NKM 16-2-4 was established as a carrier for M cell-targeted mucosal vaccines and can be used for detection of M cells.

1.2 Applications

- Identification and enumeration of villous M cells by flow cytometry or fluorescence microscopy.
- Antibody-mediated vaccination studies.¹
- Immunohistochemistry (paraffin or frozen sections).¹

1.3 Recommended antibody dilution

The recommended antibody dilution for all Anti-Microfold (M) Cell conjugates is **1:11 for up to 10⁷ cells/100 µL** of buffer for labeling of cells and analysis by flow cytometry.

The antibody is suited for staining of formaldehyde-fixed cells. Cells should be stained prior to fixation, if formaldehyde is used as a fixative.

1.4 Reagent requirements

- Buffer: Prepare a phosphate-buffered solution containing Ca²⁺ and Mg²⁺, pH 7.2, e.g., Hanks' Balanced Salt Solution (HBSS): 138 mM NaCl, 5.3 mM KCl, 0.2 mM Na₂HPO₄, 0.4 mM KH₂PO₄, 1.3 mM CaCl₂, 0.8 mM MgSO₄, 4.2 mM NaHCO₃. Add 0.5% bovine serum albumine (BSA). Keep buffer cold (2–8 °C).
- (Optional) FcR Blocking Reagent, mouse (# 130-092-575) to avoid Fc receptor-mediated antibody labeling.
- (Optional) Anti-PE MicroBeads (# 130-048-801), or Anti-APC MicroBeads (# 130-090-855) for subsequent indirect magnetic labeling.
- (Optional) CD45-FITC (# 130-091-609), CD45-VioBlue® (# 130-092-910), CD45-PerCP (# 130-094-962), CD45-PE (# 130-091-610), or CD45-APC (# 130-091-811). For more information about antibodies refer to www.miltenyibiotec.com/antibodies.
- (Optional) Propidium Iodide Solution (# 130-093-233) or 7-AAD for flow cytometric exclusion of dead cells without fixation.
- (Optional) Fixation and Dead Cell Discrimination Kit (# 130-091-163) for cell fixation and flow cytometric exclusion of dead cells.

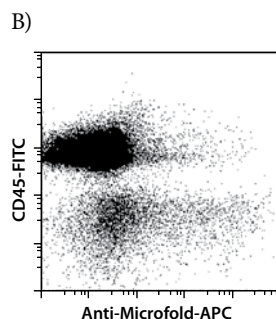
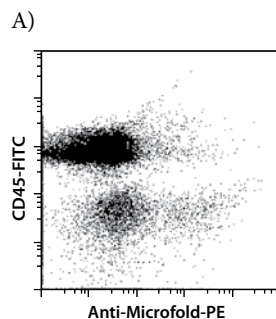
2. General protocol for immunofluorescent staining

▲ Volumes given below are for up to 10^7 nucleated 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 nucleated cells, use twice the volume of all indicated reagent volumes and total volumes).

1. Determine cell number.
2. Centrifuge cell suspension at $300 \times g$ for 10 minutes. Aspirate supernatant completely.
3. Resuspend up to 10^7 nucleated cells per 100 μ L of buffer.
4. Add 10 μ L of the Anti-Microfold (M) Cell antibody.
5. Mix well and 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 $300 \times g$ for 10 minutes. Aspirate supernatant completely.
7. Resuspend cell pellet in a suitable amount of buffer for analysis by flow cytometry or fluorescence microscopy.

3. Examples of immunofluorescent staining with Anti-Microfold (M) Cell antibodies

Murine Peyer's Patches (PP) were stained with Anti-Microfold (M) Cell antibodies conjugated to PE (A) or APC (B) as well as with CD45-FITC (# 130-091-609) and analyzed by flow cytometry using the MACSQuant® Analyzer. Cell debris and dead cells were excluded from the analysis based on scatter signals and propidium iodide fluorescence.



4. References

1. Nochi, T. *et al.* (2007) A novel M cell – specific carbohydrate targeted mucosal vaccine effectively induces antigen-specific immune responses. *J. Exp. Med.* 204: 2789–2796.
2. Terahara, K. *et al.* (2008) Comprehensive gene expression profiling of peyer's patch M cells, villous M-like cells, and intestinal epithelial cells. *J. Immunol.* 180: 7840–7846.
3. Kim, S-H. *et al.* (2010) The M cell-targeting ligand promotes antigen delivery and induces antigen-specific immune responses in mucosal vaccination. *J. Immunol.* 185: 5787–5795.
4. Kim, D-Y. *et al.* (2011) The airway antigen sampling system: respiratory M Cells as an alternative gateway for inhaled antigens. *J. Immunol.* 186: 4253–426.

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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