

# CD206 antibodies

## human

CD206-FITC	130-095-131
CD206-PE	130-095-220
CD206-APC	130-095-217
CD206-Biotin	130-095-214

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## 1. Description

<b>Components</b>	1 mL CD206 antibodies, human: monoclonal CD206 antibodies conjugated to fluorescein isothiocyanate (FITC), R-phycoerythrin (PE), allophycocyanin (APC), or biotin.
<b>Clone</b>	DCN228 (isotype: mouse IgG1).
<b>Capacity</b>	100 tests or up to 10 <sup>9</sup> total cells.
<b>Product format</b>	Antibodies are supplied in buffer containing stabilizer and 0.05% sodium azide.
<b>Storage</b>	Store protected from light at 2–8 °C. Do not freeze. The expiration date is indicated on the vial label.

### 1.1 Background information

The mannose receptor (MR), also known as macrophage MR or CD206, is a 162-175 kD type I membrane protein. It contains an N-terminal, cystein-rich domain, and eight C-type lectin-like domains (CTLDs), beside a transmembrane and a cytoplasmic domain. CD206 is expressed by macrophages, dendritic cells, and subsets of endothelial cells, but not on monocytes. The MR recognizes multiple mainly microbial carbohydrates with mannose, fucose, or N-acetyl glucosamine residues. The MR mediates endocytosis and phagocytosis, linked to antigen presentation. It plays an important role in host defense and provides a link between innate and adaptive immunity.<sup>1-7</sup>

### 1.2 Applications

- Identification and enumeration of CD206<sup>+</sup> cells by flow cytometry or fluorescence microscopy.

### 1.3 Recommended antibody dilution

For antibody labeling of human cells.

CD206 conjugate	FITC	PE	APC	Biotin
Flow cytometry <sup>a</sup>				
- In general	1:11	1:11	1:11	1:11
- Formaldehyde-fixed cells <sup>b</sup>	1:11	1:11	1:11	1:11
Immunohistochemistry <sup>c</sup>				
	1:11	1:11	1:11	1:11

- a) The indicated antibody dilutions are for up to 10<sup>7</sup> cells/100 µL of buffer.  
 b) For optimal results, cells must be stained prior to fixation.  
 c) The optimal antibody dilution should be determined by the user.

### 1.4 Reagent requirements

- 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<sup>®</sup> BSA Stock Solution (# 130-091-376) 1:20 with autoMACS<sup>®</sup> Rinsing Solution (# 130-091-222). Keep buffer cold (2–8 °C).
  - ▲ **Note:** EDTA can be replaced by other supplements such as anticoagulant citrate dextrose formula-A (ACD-A) or citrate phosphate dextrose (CPD). BSA can be replaced by other proteins such as human serum albumin, human serum, or fetal bovine serum (FBS). Buffers or media containing Ca<sup>2+</sup> or Mg<sup>2+</sup> are not recommended for use.
- (Optional) FcR Blocking Reagent, human (# 130-059-901) to avoid Fc receptor-mediated antibody labeling.
- (Optional) Anti-Biotin-VioBlue<sup>®</sup> (# 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 CD206-Biotin.
- (Optional) Anti-FITC MicroBeads (# 130-048-701), Anti-PE MicroBeads (# 130-048-801), Anti-APC MicroBeads (# 130-090-855), or Anti-Biotin MicroBeads (# 130-090-485) for subsequent indirect magnetic labeling.
- (Optional) CD11c-FITC (# 130-092-410) or CD11c-PE (# 130-092-411). For more information about antibodies refer to [www.miltenyibiotec.com](http://www.miltenyibiotec.com).
- (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.

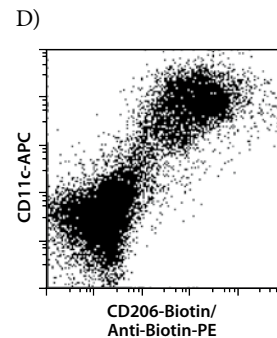
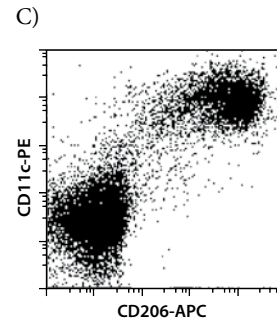
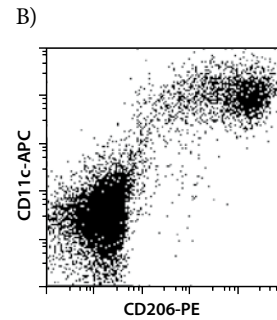
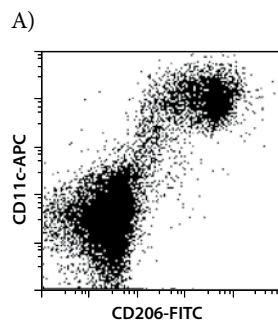
## 2. General protocol for immunofluorescent staining

▲ 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  $2 \times 10^7$  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  $\mu$ L of buffer.
4. Add 10  $\mu$ L of the CD206 antibody.
5. Mix well and incubate for 10 minutes in the dark in the refrigerator ( $2-8^\circ\text{C}$ ).  
▲ **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. (Optional) If CD206-Biotin was used, resuspend the cell pellet in 100  $\mu$ L of buffer, add 10  $\mu$ L of anti-biotin antibody (Anti-Biotin-VioBlue, Anti-Biotin-FITC, Anti-Biotin-PE, or Anti-Biotin-APC), 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 CD206 antibodies

Human peripheral blood mononuclear cells (PBMCs) were cultured in the presence of GM-CSF for 6 days. Cells were stained with CD206 antibodies conjugated to FITC (A), PE (B), or APC (C), as well as with CD11c-PE (#130-092-411) or CD11c-APC (#130-092-412) and analyzed by flow cytometry using the MACSQuant® Analyzer. Cells labeled with CD206-Biotin (D) were stained with Anti-Biotin-APC (#130-090-856) as well as CD11c-PE. Cell debris and dead cells were excluded from the analysis based on scatter signals and propidium iodide fluorescence.



## 4. References

1. Sallusto, F. *et al.* (1995) Dendritic cells use macropinocytosis and the mannose receptor to concentrate macromolecules in the major histocompatibility complex class II compartment: downregulation by cytokines and bacterial products. *J. Exp. Med.* 182: 389–400.
2. Kang, P. B. *et al.* (2005) The human macrophage mannose receptor directs Mycobacterium tuberculosis lipoarabinomannan-mediated phagosome biogenesis. *J. Exp. Med.* Vol. 202: 987–999.
3. Feinberg, H. *et al.* (2000) Structure of a C-type carbohydrate recognition domain from the macrophage mannose receptor. *J. Biol. Chem.* 275: 21539–21548.
4. Tacke, P. J. *et al.* (2005) Effective induction of naive and recall T-cell responses by targeting antigen to human dendritic cells via a humanized anti-DC-SIGN antibody. *Blood* 106: 1278–1285.
5. Ezekowitz, R. A. B. *et al.* (1990) Molecular characterization of the human macrophage mannose receptor: demonstration of multiple carbohydrate recognition-like domains and phagocytosis of yeasts in Cos-1 cells. *J. Exp. Med.* 172: 1785–1794.
6. Lennartz, M. R. *et al.* (1989) Biosynthesis and processing of the mannose receptor in human macrophages. *J. Biol. Chem.* 264: 2385–2390.
7. Wileman, T. E. *et al.* (1986) Identification of the macrophage mannose receptor as a 175-kDa membrane protein. *Proc. Natl. Acad. Sci. USA* 83: 2501–2505.

All protocols and data sheets are available at [www.miltenyibiotec.com](http://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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