

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. Example of immunofluorescent staining with CD205 antibodies
4. References

1. Description

| | | | | | | | | | |
|-----------------------|--|----|-------------|-----|-------------|--------|-------------|------|-------------|
| Components | 1 mL monoclonal CD205 antibodies, human conjugated to: | | | | | | | | |
| | <table border="0"> <tr> <td>PE</td> <td>130-096-369</td> </tr> <tr> <td>APC</td> <td>130-096-378</td> </tr> <tr> <td>Biotin</td> <td>130-096-447</td> </tr> <tr> <td>pure</td> <td>130-096-439</td> </tr> </table> | PE | 130-096-369 | APC | 130-096-378 | Biotin | 130-096-447 | pure | 130-096-439 |
| PE | 130-096-369 | | | | | | | | |
| APC | 130-096-378 | | | | | | | | |
| Biotin | 130-096-447 | | | | | | | | |
| pure | 130-096-439 | | | | | | | | |
| Clone | HD30 (isotype: mouse IgG1). | | | | | | | | |
| Capacity | 100 tests or up to 10 ⁹ total cells. The unconjugated (pure) antibody is supplied at a concentration of 100 µg/mL. | | | | | | | | |
| 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 CD205 antibody has been tested to react with rhesus monkey (*Macaca mulatta*) cells.

1.1 Background information

Clone HD30 detects the CD205 glycoprotein. CD205 (also known as DEC-205 or Ly-75) is a 210 kD C-type lectin single-pass membrane protein (type I), which is highly expressed on thymic epithelial cells and dendritic cells¹. Unlike murine CD205, human CD205 is also found at low levels on other peripheral blood lymphocytes, eg. monocytes, T-cells, B-cells and NK-cells. CD205 belongs to the macrophage mannose receptor family and acts as an endocytic receptor to uptake antigens, direct the captured antigens from the extracellular space to the antigen-processing compartment for antigen presentation on MHC class II and cross-presentation on MHC class I molecules^{2,3}. Another function of CD205 is the clearance of apoptotic cells, potentially an important pathway for the uptake of self-antigen in intrathymic and peripheral tolerance⁴.

1.2 Applications

- Identification and enumeration of CD205⁺ cells by flow cytometry.

1.3 Recommended antibody dilution

The recommended antibody dilution for all CD205 conjugates is **1:11 for up to 10⁷ cells/100 µL** of buffer for labeling of cells and subsequent analysis by flow cytometry.

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

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[®] BSA Stock Solution (# 130-091-376) 1:20 with autoMACS[®] 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 Ca²⁺ or Mg²⁺ are not recommended for use.
- (Optional) FcR Blocking Reagent, human (# 130-059-901) to avoid Fc receptor-mediated antibody labeling.
- (Optional) Anti-Biotin antibodies conjugated to, e.g., PE (# 130-090-756) as secondary antibody reagent in combination with CD205-Biotin.
- (Optional) CD11c-PE (# 130-092-411) or CD11c-APC (# 130-092-412). For more information about antibodies refer to www.miltenyibiotec.com/antibodies.
- (Optional) Mouse IgG1 isotype control antibodies conjugated to, e.g., Mouse IgG1 (# 130-092-212). For more information about isotype control antibodies refer to www.miltenyibiotec.com.
- (Optional) Propidium Iodide Solution (# 130-093-233) for flow cytometric exclusion of dead cells without fixation.

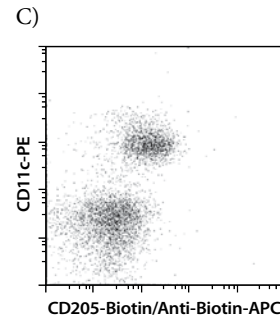
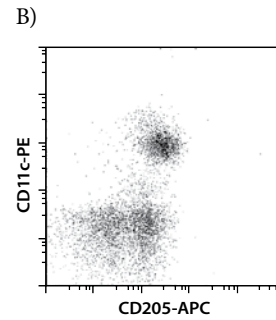
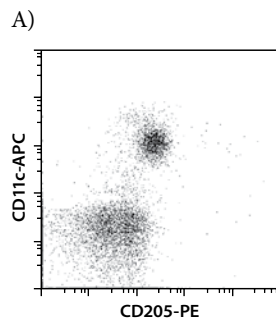
2. General protocol for immunofluorescent staining

▲ 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 CD205 antibody.
▲ **Note:** Refer to section 1.3 for exceptions.
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 CD205-Biotin was used, resuspend the cell pellet in 100 μ L of buffer, add 10 μ L 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. Example of immunofluorescent staining with CD205 antibodies

Human peripheral blood mononuclear cells (PBMCs) were stained with CD205 antibodies conjugated to PE (A) or APC (B) 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 CD205-Biotin (C) were stained with Anti-Biotin-APC (# 1130-090-856) 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. Heath, W. *et al.* (2004) Cross-presentation, dendritic cell subsets, and the generation of immunity to cellular antigens. *Immunol. Rev.* 199: 9–26.
2. Bonifaz, L. *et al.* (2004) *In vivo* targeting of antigens to maturing dendritic cells via the DEC-205 receptor improves T cell vaccination. *J. Exp. Med.* 199: 815–824.
3. Guo, M. *et al.* (2000) A monoclonal antibody to the DEC-205 endocytosis receptor on human dendritic cells. *Hum. Immunol.* 61(8): 729–738.
4. Shrimpton, R. *et al.* (2009) CD205 (DEC-205): A recognition receptor for apoptotic and necrotic self. *Mol Immunol.* 46(6): 1229–1239.

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.

Warranty

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