Miltenyi Biotec

CD115 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 CD115 antibodies
4. References

1. Description

Components 1 mL monoclonal CD115 antibodies, mouse conjugated to various dyes.

PE	130-096-308
APC	130-096-310
Biotin	130-096-323

Clone AFS98 (isotype: rat IgG2a).

Capacity 100 tests or up to 10^9 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.

1.1 Background information

Clone AFS98 binds the protein tyrosine kinase transmembrane receptor for macrophage colony-stimulating factor (M-CSF), also known as CSF-1 as well as interleukin 34 (IL-34). CD115 is a single-pass type I membrane protein and receptor activation induces homo-dimerization which leads to phosphorylation and ubiquitinylation of intracellular residues. It is expressed on monocytes, macrophages, and osteoclasts as well as on common dendritic cell precursors (CDP) and macrophage/dendritic cell precursors (MDP).

1.2 Applications

- Identification and enumeration of $CD115^+$ cells by flow cytometry or fluorescence microscopy.
- Differentiation studies of monocytes and precursors.
- MACS® Control experiment in combination with the CD115 MicroBead Kit, mouse (# 130-096-354).

  ▲ **Note:** It is recommended to use CD115-PE or CD115-APC as control reagent.

1.3 Recommended antibody dilution

The recommended antibody dilution for all CD115 conjugates is **1:11 for up to 10^7 cells/100 µL** of buffer for labeling of cells and analysis by flow cytometry. For CD115 MicroBead-labeled cells use the same dilution.

The antibody is not 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 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 mouse serum albumin, mouse serum, or fetal bovine serum (FBS). Buffers or media containing Ca^{2+} or Mg^{2+} are not recommended for use.
- (Optional) FcR Blocking Reagent, mouse (# 130-092-575) 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 CD115-Biotin.
- (Optional) Anti-Ly-6C-FITC (# 130-093-134), Anti-Ly-6C-PE (# 130-093-135), or Anti-Ly-6C-APC (# 130-093-136). 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×g for 10 minutes. Aspirate supernatant completely.
3. Resuspend up to 10^7 nucleated cells per 100 µL of buffer.
4. Add 10 µL of the CD115 antibody.

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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.

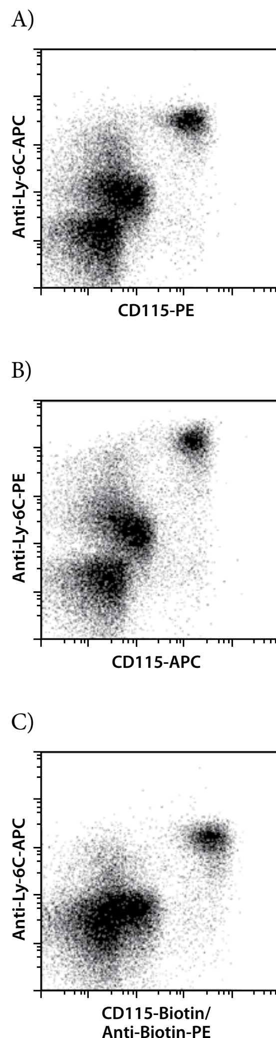
6. Wash cells by adding 1–2 mL of buffer and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.

7. (Optional) If CD115-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 CD115 antibodies

Mouse bone marrow cells were stained with Anti-CD115 antibodies conjugated to PE (A), or APC (B) as well as with Anti-Ly-6C-PE (# 130-093-135) or Anti-Ly-6C-APC (# 130-093-136) and analyzed by flow cytometry using the MACSQuant® Analyzer. Cells labeled with CD115-Biotin (C) were stained with Anti-Biotin-PE (# 130-090-756) as well as Anti-Ly-6C-APC. Cell debris and dead cells were excluded from the analysis based on scatter signals and propidium iodide fluorescence.



4. References

1. Sudo, T. *et al.* (1995) Functional hierarchy of c-kit and c-fms in intramarrow production of CFU-M. Oncogene 11: 2469–2476.
2. Murayama, T. *et al.* (1999) Intraperitoneal administration of anti-c-fms monoclonal antibody prevents initial events of atherogenesis but does not reduce the size of advanced lesions in apolipoprotein E-deficient mice. Circulation 99: 1740–1746.
3. Sunderkötter, C. *et al.* (2004) Subpopulations of mouse blood monocytes differ in maturation stage and inflammatory response. J. Immunol. 172: 4410–4417.
4. Auffray, C. *et al.* (2009) CX_3 CR1⁺ CD115⁺ CD135⁺ common macrophage/DC precursors and the role of CX_3 CR1 in their response to infl ammation. J. Exp. Med. 206: 595–606.

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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