Anti-F4/80 antibodies, mouse

For research use only

9 µg equal 60 tests, 30 µg equal 200 tests. One test corresponds to labeling of 10^6 cells.

Product	Content	Order no.
Anti-F4/80-FITC	9 µg in 300 µL	130-102-988
Anti-F4/80-FITC	30 µg in 1 mL	130-102-327
Anti-F4/80-PE	9 µg in 300 µL	130-102-943
Anti-F4/80-PE	30 µg in 1 mL	130-102-422
Anti-F4/80-APC	9 µg in 300 µL	130-102-942
Anti-F4/80-APC	30 µg in 1 mL	130-102-379
Anti-F4/80-PE-Vio615	9 µg in 300 µL	130-107-744
Anti-F4/80-PE-Vio615	30 µg in 1 mL	130-107-709
Anti-F4/80-PE-Vio770	9 µg in 300 µL	130-102-941
Anti-F4/80-PE-Vio770	30 µg in 1 mL	130-102-193
Anti-F4/80-PerCP-Vio700	9 µg in 300 µL	130-102-940
Anti-F4/80-PerCP-Vio700	30 µg in 1 mL	130-102-161
Anti-F4/80-Biotin	9 µg in 300 µL	130-102-029
Anti-F4/80-Biotin	30 µg in 1 mL	130-101-893

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.

Technical data and background information

Antigen	F4/80
Clone	REA126
Isotype	recombinant human IgG1
Isotype control	REA Control antibodies
Alternative names of antigen	ADGRE1, DD7A5-7, EGF-TM7, EMR1, Gpf480, Ly71, TM7LN3
Molecular mass of antigen [kDa]	99
Distribution of antigen	macrophages
Product format	Antibodies are supplied in buffer containing stabilizer and 0.05% sodium azide.
Fixation	Cells should be stained prior to fixation, if formaldehyde is used as a fixative.
Storage	Store protected from light at 2–8 °C. Do not freeze.

Clone REA126 recognizes F4/80, a member of epidermal growth factor (EGF)-transmembrane 7 (TM7) family. F4/80 is considered as one of the most specific cell-surface markers for murine macrophages. F4/80 is a seven-span transmembrane molecule with a large extracellular, multiple EGF module containing, domain. Constitutive and high expression of F4/80 is found on most resident tissue macrophages, including the spleen, microglia in the brain, Kupffer's cells in the liver, and Langerhans cells in the skin. In addition, the expression of F4/80 can also be regulated depending on the physiological status of the cell. F4/80 molecule is required for the differentiation of antigen-specific $CD8^+$ Tregs and is involved in inducing peripheral immune tolerance. No specific ligand for F4/80 has been reported so far.
Additional information: Clone REA126 displays negligible binding to Fc receptors.

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). Buffers or media containing Ca^{2+} or Mg^{2+} are not recommended for use.
- (Optional) Fluorochrome-conjugated anti-biotin antibodies, e.g., Anti-Biotin-PE (# 130-090-756) as secondary antibody reagent in combination with biotinylated antibodies.
- (Optional) Propidium Iodide Solution (# 130-093-233) 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.

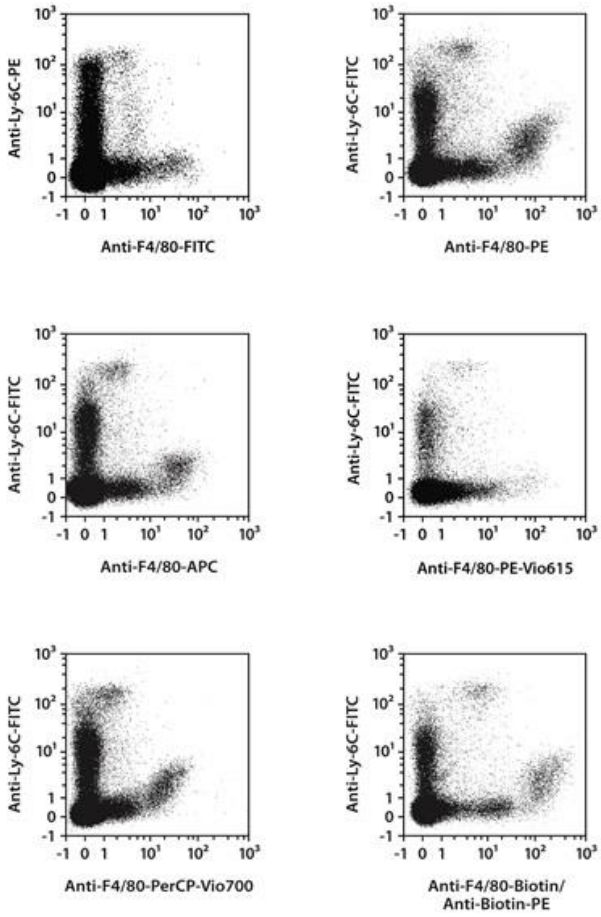
Protocol for cell surface staining

- The recommended antibody dilution for labeling of cells and subsequent analysis by flow cytometry is 1:10 for up to 10^6 cells/50 µL of buffer.
- 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. Resuspend up to 10^6 nucleated cells per 45 µL of buffer.
4. Add 5 µL of the 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×g for 10 minutes. Aspirate supernatant completely.
7. (Optional) If biotinylated antibody was used, resuspend the cell pellet in 100 µL of buffer, add 10 µL of fluorochrome-conjugated 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.

Examples of immunofluorescent staining

Spleen cells from BALB/c mouse were stained with Anti-F4/80 antibodies as well as with Anti-Ly-6C antibodies and analyzed by flow cytometry using the MACSQuant® Analyzer. $CD45.2^+$ cells were pre-gated for the analysis. Cell debris and dead cells were excluded from the analysis based on scatter signals and propidium iodide fluorescence or 4',6-diamidino-2-phenylindole (DAPI) fluorescence, as in the case of tandems.



References

1. **Lin, H-H. *et al.*** (2005) The macrophage F4/80 receptor is required for the induction of antigen-specific efferent regulatory T cells in peripheral tolerance. J. Exp. Med. 201: 1615–1625.
2. **Gordon, S. *et al.*** (2011) F4/80 and the related adhesion-GPCRs. Eur. J. Immunol. 41(9): 2472–2476.
3. **Hume, D. A. *et al.*** (1984) The mononuclear phagocyte system of the mouse defined by immunohistochemical localization of antigen F4/80: macrophages of bone and associated connective tissue. J. Cell. Sci. 66: 189–194.

Warranty

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