CD80 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.
CD80-FITC	9 µg in 300 µL	130-102-882
CD80-FITC	30 µg in 1 mL	130-102-532
CD80-PE	9 µg in 300 µL	130-102-883
CD80-PE	30 µg in 1 mL	130-102-613
CD80-APC	9 µg in 300 µL	130-102-884
CD80-APC	30 µg in 1 mL	130-102-584
CD80-PE-Vio770	9 µg in 300 µL	130-102-885
CD80-PE-Vio770	30 µg in 1 mL	130-102-372
CD80-Biotin	9 µg in 300 µL	130-102-010
CD80-Biotin	30 µg in 1 mL	130-101-953

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	CD80
Clone	16-10A1
Isotype	hamster IgG2
Alternative names of antigen	B7-1, Cd28l, Ly-53, Ly53, MIC17, TSA1, B7, BB1
Molecular mass of antigen [kDa]	30
Cross-reactivity	dog
Distribution of antigen	B cells, dendritic cells, Langerhans cells, macrophages, monocytes, T cells
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.

The monoclonal antibody 16-10A1 recognizes mouse CD80 also known as B7, B7-1 or Ly-53. The 55 kDa member of the Ig superfamily is expressed on activated B cells, macrophages and dendritic cells. The interaction of CD80 and CD152 (CTLA-4) or CD28 induces T cell proliferation and cytokine production.

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) FcR Blocking Reagent, mouse (# 130-092-575) to avoid Fc receptor–mediated antibody labeling.
- (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

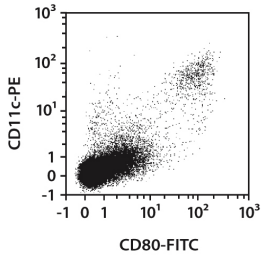
- The recommended antibody dilution for labeling of cells and subsequent analysis by flow cytometry is 1:10 for up to 10^6 cells/50 µL of buffer.
- Volumes given below are for up to 10^6 nucleated cells. When working with fewer than 10^6 cells, use the same volumes as indicated. When working with higher 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.

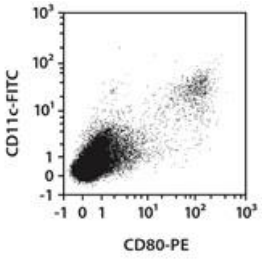
Examples of immunofluorescent staining

LPS activated mouse splenocytes were stained with CD80 antibodies conjugated to (A) FITC (B) PE (C) APC (D) PE-Vio770 and (E) Biotin, as well as with CD11c antibodies conjugated to FITC or PE and analyzed by flow cytometry using the MACSQuant® Analyzer. Cells labeled with CD80-Biotin were stained with Anti-Biotin-PE as well. 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 CD80-PE-Vio770.

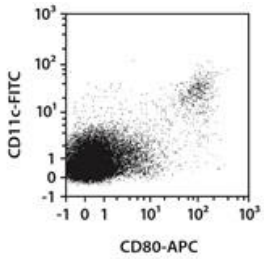
A:



B:



C:



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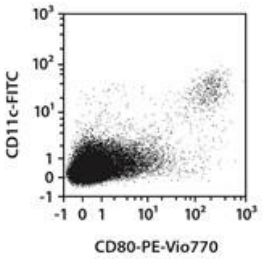

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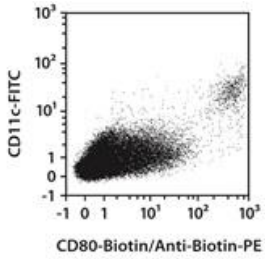

References

1. **Bluestone, J. A. *et al.*** (1995) New perspectives of CD28-B7-mediated T cell costimulation. Immunity 2(6): 555–559.
2. **Hathcock, K. S *et al.*** (1994) Comparative analysis of B7-1 and B7-2 costimulatory ligands: expression and function. J. Exp. Med. 180: 631.
3. **Harlan, D. M. *et al.*** (1994) Mice expressing both B7-1 and viral glycoprotein on pancreatic beta cells along with glycoprotein-specific transgenic T cells develop diabetes due to a breakdown of T-lymphocyte unresponsiveness. Proc. Natl. Acad. Sci. U.S.A. 91: 3137–3141.
4. **Razi-Wolf, Z. *et al.*** (1992) Expression and function of the murine B7 antigen, the major costimulatory molecule expressed by peritoneal exudate cells. Proc. Natl. Acad. Sci. U.S.A. 89: 4210–4214.

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