CD86 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.
CD86-FITC	30 µg in 1 mL	130-102-506
CD86-PE	30 µg in 1 mL	130-102-604
CD86-APC	30 µg in 1 mL	130-102-558
CD86-VioBlue	30 µg in 1 mL	130-102-438
CD86-PE-Vio770	9 µg in 300 µL	130-105-195
CD86-PE-Vio770	30 µg in 1 mL	130-105-135
CD86-APC-Vio770	9 µg in 300 µL	130-105-196
CD86-APC-Vio770	30 µg in 1 mL	130-105-136
CD86-PerCP-Vio700	9 µg in 300 µL	130-105-197
CD86-PerCP-Vio700	30 µg in 1 mL	130-105-137
CD86-Biotin	30 µg in 1 mL	130-101-944

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	CD86
Clone	PO3.3
Isotype	rat IgG2bκ
Isotype control	Rat IgG2b – isotype control antibodies
Alternative names of antigen	B7-2, B70, CLS1, Cd28l2, ETC-1, Ly-58, MB7-2, TS/A-2
Molecular mass of antigen [kDa]	32
Distribution of antigen	B cells, dendritic cells, endothelial cells, Langerhans cells, monocytes, T cells
Product format	Antibodies are supplied in buffer containing stabilizer and 0.05% sodium azide.
Fixation	The antibody is suited for staining of formaldehyde-fixed cells.
Storage	Store protected from light at 2–8 °C. Do not freeze.

Antibody clone PO3.3 detects the CD86 antigen, also known as B7-2, an 80 kDa molecule and a member of the immunoglobulin superfamily. Together with CD80 (B7-1) it belongs to the B7 family of costimulatory molecules. CD86 is expressed on activated antigen-presenting cells, including B cells,

dendritic cells, and monocytes/macrophages. The interaction of CD86 with its ligands CD28 and CD152 (CTLA- 4) plays a critical role in induction and regulation of immune responses, e.g., cross-talk between T and B cells, T cell costimulation, or immunoglobulin class-switching. Binding of CD86 to CD28 on T cells results in transduction of costimulatory signals for activation or proliferation of T cells, or cytokine production. In contrast, binding of CD86 to CTLA-4 regulates T cell activation and diminishes the immune response.

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.

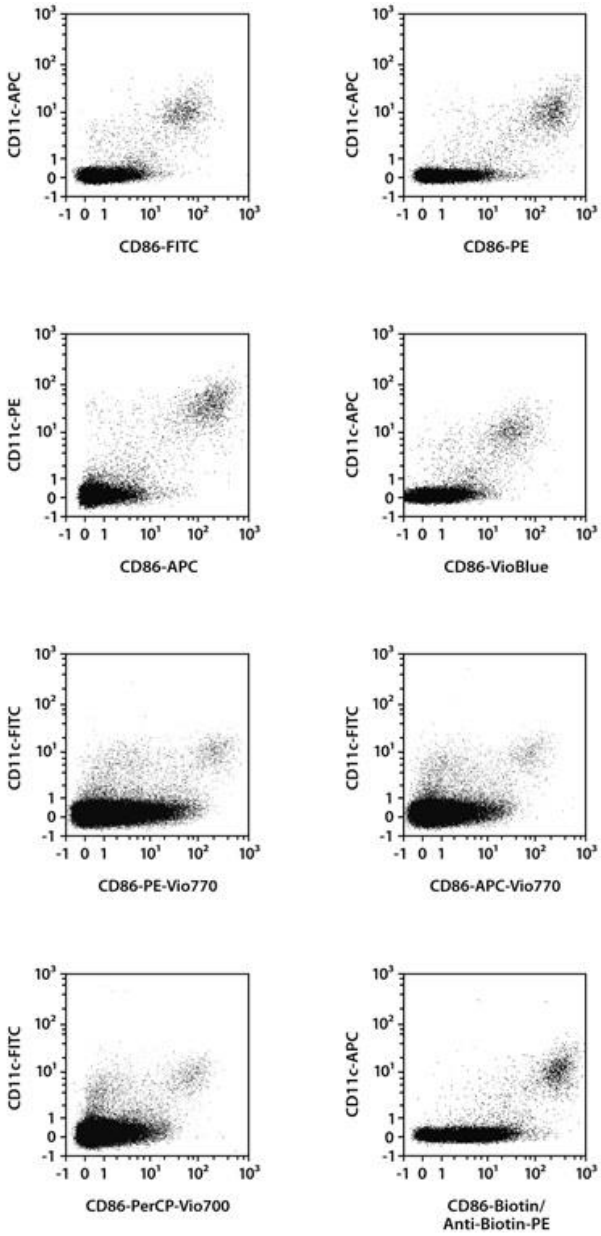
Protocol for cell surface staining

- 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

BALB/c splenocytes spiked with LPS-stimulated $CD11c^+$ dendritic cells were stained with CD86 antibodies as well as with CD11c-APC, -PE or -FITC and analyzed by flow cytometry using the MACSQuant® Analyzer. Cell debris and dead cells were excluded from the analysis based on scatter signals and propidium iodide fluorescence.



References

1. **Nakajima, A. *et al.*** (1997) Requirement of CD28-CD86 co-stimulation in the interaction between antigen-primed T helper type 2 and B cells. Int. Immunol. 9(5): 637–644.
2. **Kin, N. W. *et al.*** (2006) CD86 stimulation on a B cell activates the phosphatidylinositol 3-kinase/Akt and phospholipase C gamma 2/protein kinase C alpha beta signaling pathways. J. Immunol. 176(11): 6727–6735.
3. **Zhu, X. Y. *et al.*** (2005) Blockade of CD86 signaling facilitates a Th2 bias at the maternal-fetal interface and expands peripheral CD4+CD25+ regulatory T cells to rescue abortion-prone fetuses. Biol. Reprod. 72(2): 338–345.

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