CD86 antibodies, rat

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-VioBright FITC	9 µg in 300 µL	130-109-180
CD86-VioBright FITC	30 µg in 1 mL	130-109-133
CD86-PE	9 µg in 300 µL	130-109-176
CD86-PE	30 µg in 1 mL	130-109-129
CD86-APC	9 µg in 300 µL	130-109-177
CD86-APC	30 µg in 1 mL	130-109-130
CD86-PE-Vio770	9 µg in 300 µL	130-109-178
CD86-PE-Vio770	30 µg in 1 mL	130-109-131
CD86-APC-Vio770	9 µg in 300 µL	130-109-179
CD86-APC-Vio770	30 µg in 1 mL	130-109-132
CD86-Biotin	9 µg in 300 µL	130-109-175
CD86-Biotin	30 µg in 1 mL	130-109-128

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	24F
Isotype	mouse IgG1κ
Isotype control	Mouse IgG1 – isotype control antibodies
Alternative names of antigen	B7-2, B70
Molecular mass of antigen [kDa]	32
Distribution of antigen	B cells, dendritic cells, macrophages, monocytes
Product format	Reagents 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 24F recognizes the rat antigen CD86, also known as B7-2, which is a member of the B7 family of costimulatory molecules. In addition to CD80 (B7-1) , CD86 is a counter-receptor for the T cell surface

molecules CD28 and CD152 (CTLA-4). It is expressed by germinal center B cells and weakly expressed on antigen presenting cells, such as macrophages and dendritic cells. Its expression is upregulated on B cells through a variety of surface stimuli including LPS. CD86 plays a critical role in the early events of T cell activation and costimulation of naive T cells as well as autoantibody and Th2-mediated Ig 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.

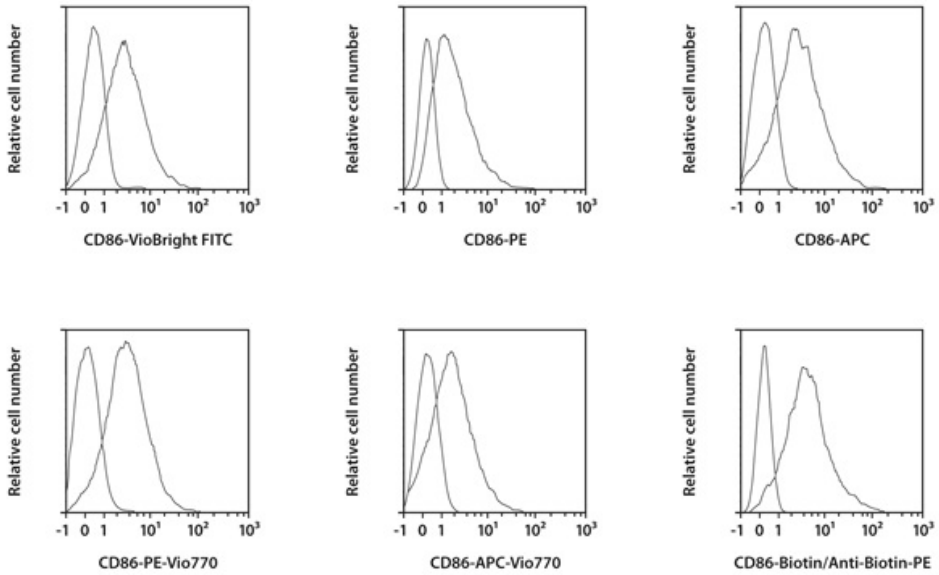
Protocol for cell surface staining

- 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

Splenocytes from LOU rats were stimulated for 3 days with LPS. $CD45R^+$ cells were pregated for the analysis and stained with CD86 antibodies or with the corresponding isotype control (left peak). Flow cytometry was performed using the MACSQuant® Analyzer. Cell debris and dead cells were excluded from the analysis based on scatter signals and propidium iodide fluorescence.



References

1. **Bluestone, J. A. *et al.*** (1995) New perspectives of CD28-B7-mediated T cell costimulation. Immunity 2(6): 555–559.
2. **Maeda, K. *et al.*** (1997) Characterization of rat CD80 and CD86 by molecular cloning and mAb. Int. Immunol. 9(7): 993–1000.
3. **Damoiseaux, J. G. *et al.*** (1998) Costimulatory molecules CD80 and CD86 in the rat; tissue distribution and expression by antigen-presenting cells. J. Leukoc. Biol. 64(6): 803–809.

Warranty

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