CD25 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.
CD25-VioBright FITC	9 µg in 300 µL	130-104-324
CD25-VioBright FITC	30 µg in 1 mL	130-104-275
CD25-PE	9 µg in 300 µL	130-102-788
CD25-PE	30 µg in 1 mL	130-102-593
CD25-APC	9 µg in 300 µL	130-102-787
CD25-APC	30 µg in 1 mL	130-102-550
CD25-PE-Vio770	9 µg in 300 µL	130-105-419
CD25-PE-Vio770	30 µg in 1 mL	130-105-378
CD25-Biotin	9 µg in 300 µL	130-101-989
CD25-Biotin	30 µg in 1 mL	130-101-938

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	CD25
Clone	7D4
Isotype	rat IgMκ
Isotype control	Rat IgM – isotype control antibodies
Alternative names of antigen	IL2RA, IL2R, Ly-43, IL-2Rα, p55, Tac
Molecular mass of antigen [kDa]	28
Distribution of antigen	B cells, basophils, lymphocytes, macrophages, monocytes, myeloid cells, NK cells, osteoblasts, T cells, thymocytes
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 CD25 antibody recognizes the α chain of the IL-2 receptor (IL-2R). IL-2Rα associates with the β chain (CD122) and the γ chain (CD132) to form the functional IL-2R complex. IL-2Rα is expressed on $CD4^+CD25^+$ regulatory T cells, activated T and B cells, and to a lesser extent on activated dendritic cells.

It is also transiently expressed during T and B cell development. Binding of the CD25 antibody does not impede the binding of IL-2 to its receptor. In combination with CD4, CD25 antibody can be used to identify regulatory T cells (Tregs).

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.

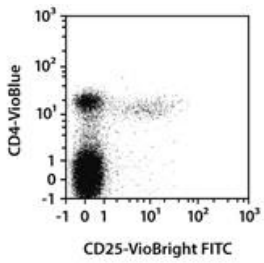
Protocol for cell surface staining

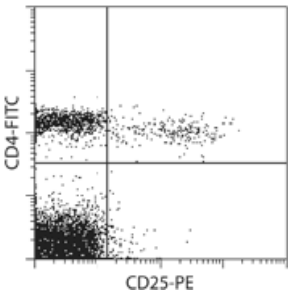
- The recommended antibody dilution for labeling of cells and subsequent analysis by flow cytometry is 1:10 for up to 10^6 cells/50 µL of buffer.
- Volumes given below are for up to 10^6 nucleated cells. When working with fewer than 10^6 cells, use the same volumes as indicated. When working with higher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

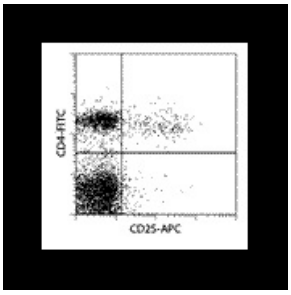
1. Determine cell number.
2. Centrifuge cell suspension at 300×g for 10 minutes. Aspirate supernatant completely.
3. Resuspend up to 10^6 nucleated cells per 45 µL of buffer.
4. Add 5 µL of the antibody.
5. Mix well and incubate for 10 minutes in the dark in the refrigerator (2−8 °C).
   Note: Higher 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.

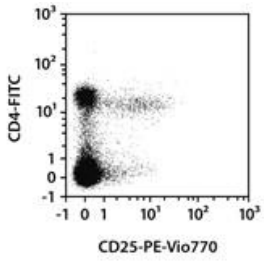
Examples of immunofluorescent staining

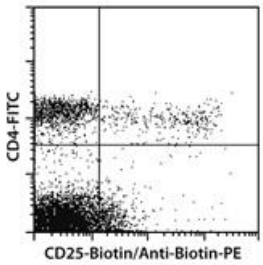
Mouse splenocytes were stained with CD25 antibodies as well as with CD4. Cells labeled with Biotin were stained with Anti-Biotin-PE in addition. Cell debris and dead cells were excluded from the analysis based on scatter signals and propidium iodide fluorescence.











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