CD3ε 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.
CD3ε-FITC	30 µg in 1 mL	130-102-270
CD3ε-PE	30 µg in 1 mL	130-102-364
CD3ε-APC	30 µg in 1 mL	130-102-314
CD3ε-VioBlue	30 µg in 1 mL	130-102-203
CD3ε-PE-Vio770	9 µg in 300 µL	130-105-504
CD3ε-PE-Vio770	30 µg in 1 mL	130-105-461
CD3ε-APC-Vio770	9 µg in 300 µL	130-105-503
CD3ε-APC-Vio770	30 µg in 1 mL	130-105-460
CD3ε-PerCP-Vio700	9 µg in 300 µL	130-105-885
CD3ε-PerCP-Vio700	30 µg in 1 mL	130-105-826
CD3ε-Biotin	30 µg in 1 mL	130-101-878

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	CD3ε
Clone	17A2
Isotype	rat IgG2bκ
Isotype control	Rat IgG2b – isotype control antibodies
Alternative names of antigen	CD3e, CD3epsilon, T3E, T3
Molecular mass of antigen [kDa]	19
Distribution of antigen	NKT cells, T cells, thymocytes
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.

The monoclonal antibody 17A2 reacts with mouse CD3ε, a part of the CD3 complex and a subunit of the TCR complex, which is expressed on all mature T lymphocytes, NKT cells, and during the development of thymocytes. Binding of 17A2 antibody to CD3 induces cell activation and proliferation.

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.

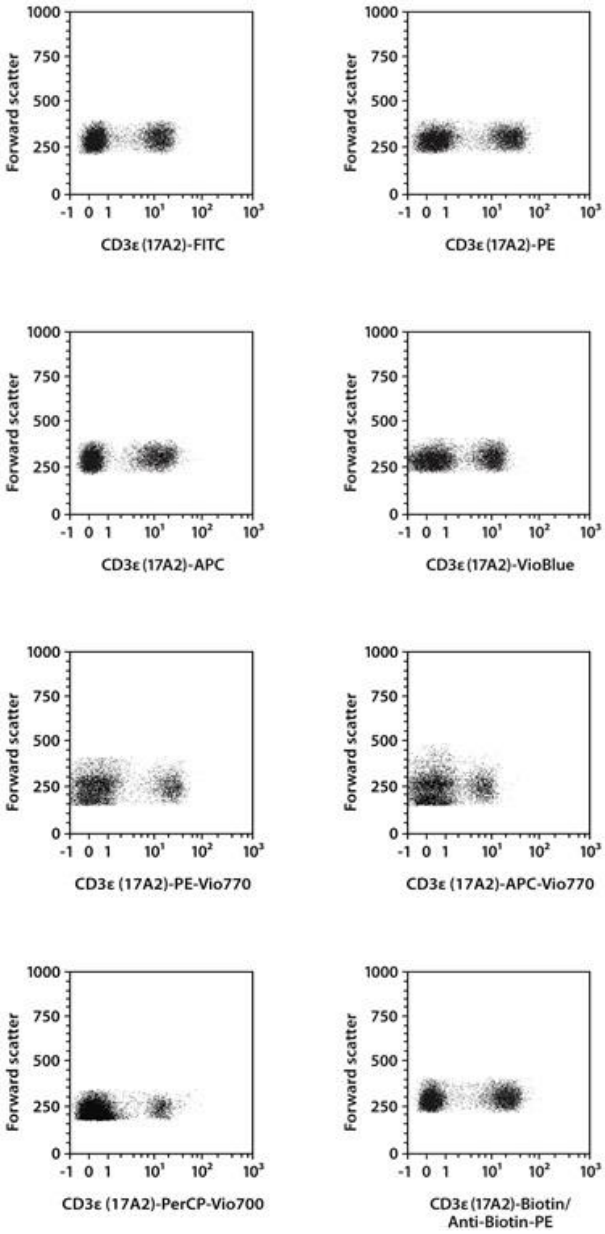
Protocol for cell surface staining

- 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 mice spleen cells were stained with CD3ε antibodies 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. **Miescher, G. C. *et al.*** (1989) Production and characterization of a rat monoclonal antibody against the murine CD3 molecular complex. Immunol. Lett. 23(2): 113–118.
2. **Exley, M. *et al.*** (1991) Structure, assembly and intracellular transport of the T cell receptor for antigen. Semin. Immunol. 3(5): 283–297.

Warranty

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