

CD3 ϵ antibodies, mouse

For research use only

9 μ g equal 60 tests, 30 μ g equal 200 tests. One test corresponds to labeling of 10^6 cells.

Product	Content	Order no.
CD3 ϵ -FITC	9 μ g in 300 μ L	130-102-791
CD3 ϵ -FITC	30 μ g in 1 mL	130-102-496
CD3 ϵ -PE	9 μ g in 300 μ L	130-102-792
CD3 ϵ -PE	30 μ g in 1 mL	130-102-600
CD3 ϵ -APC	9 μ g in 300 μ L	130-102-793
CD3 ϵ -APC	30 μ g in 1 mL	130-102-549
CD3 ϵ -VioBlue	9 μ g in 300 μ L	130-102-789
CD3 ϵ -VioBlue	30 μ g in 1 mL	130-102-441
CD3 ϵ -PE-Vio770	9 μ g in 300 μ L	130-102-794
CD3 ϵ -PE-Vio770	30 μ g in 1 mL	130-102-359
CD3 ϵ -APC-Vio770	9 μ g in 300 μ L	130-103-280
CD3 ϵ -APC-Vio770	30 μ g in 1 mL	130-102-306
CD3 ϵ -PerCP-Vio700	9 μ g in 300 μ L	130-103-855
CD3 ϵ -PerCP-Vio700	30 μ g in 1 mL	130-103-785
CD3 ϵ -Biotin	9 μ g in 300 μ L	130-101-990
CD3 ϵ -Biotin	30 μ g in 1 mL	130-101-940

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	CD3 ϵ
Clone	145-2C11
Isotype	hamster IgG1
Alternative names of antigen	CD3e, CD3epsilon, T3E, T3
Molecular mass of antigen [kDa]	19
Distribution of antigen	NK cells, 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.

Clone 145-2C11 specifically recognizes the ϵ subunit of the mouse CD3 antigen. CD3 is a 20 kDa transmembrane protein and is part of the TCR complex. Along with the γ and δ subunits of CD3, the ϵ chain is required for proper assembly and expression of the TCR complex. CD3 ϵ is expressed on thymocytes, mature T lymphocytes, and NKT cells.

Antibody clone 145-2C11 can be used for T cell activation and expansion.

Reagent requirements

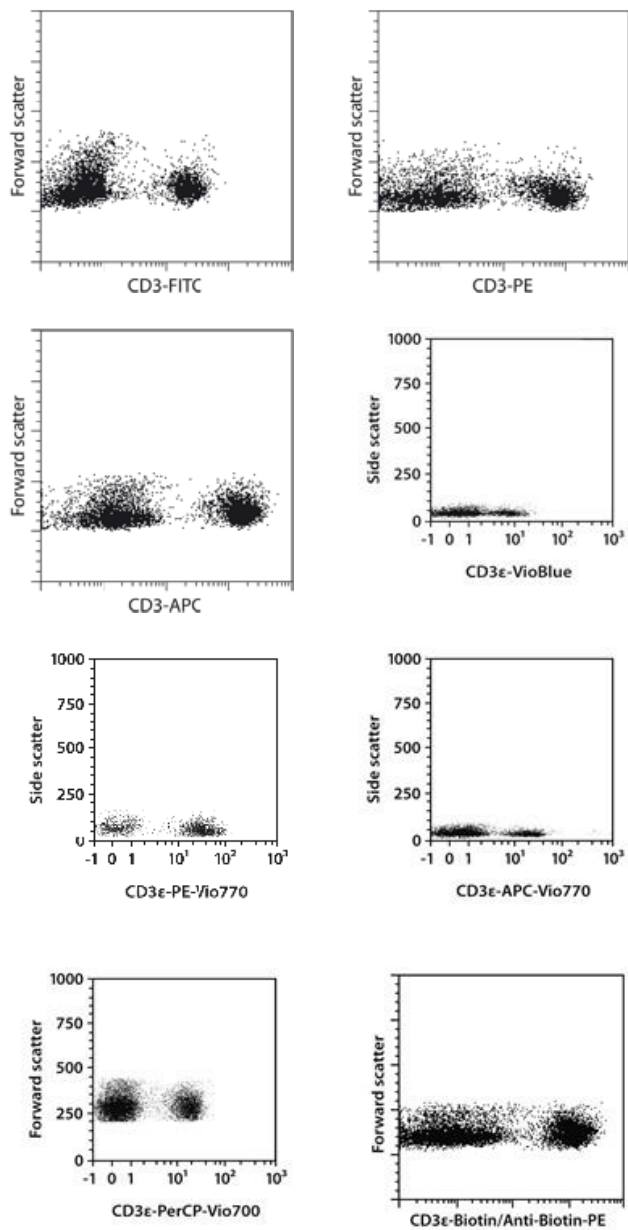
- Buffer: Prepare a solution containing phosphate-buffered saline (PBS), pH 7.2, 0.5% bovine serum albumin (BSA), and 2 mM EDTA by diluting MACS[®] BSA Stock Solution (# 130-091-376) 1:20 with autoMACS[®] Rinsing Solution (# 130-091-222). Keep buffer cold (2–8 °C).
Note: EDTA can be replaced by other supplements such as anticoagulant citrate dextrose formula-A (ACD-A) or citrate phosphate dextrose (CPD). Buffers or media containing Ca²⁺ or Mg²⁺ 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 10⁶ cells/50 μ L of buffer.
 - Volumes given below are for up to 10⁶ nucleated cells. When working with fewer than 10⁶ 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 \times 10⁶ nucleated cells, use twice the volume of all indicated reagent volumes and total volumes).
1. Determine cell number.
 2. Centrifuge cell suspension at 300 \times g for 10 minutes. Aspirate supernatant completely.
 3. Resuspend up to 10⁶ nucleated cells per 45 μ L of buffer.
 4. Add 5 μ L 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 \times g for 10 minutes. Aspirate supernatant completely.
 7. (Optional) If biotinylated antibody was used, resuspend the cell pellet in 100 μ L of buffer, add 10 μ L 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 CD3 ϵ antibodies and analyzed by flow cytometry. Cells incubated with FcR Blocking Reagent before staining with CD3 ϵ antibodies. Cells stained with CD3 ϵ -Biotin were stained with Anti-Biotin-PE in addition. Cell debris and dead cells were excluded from the analysis based on scatter signals and PI fluorescence or 4',6-diamidino-2-phenylindole (DAPI) fluorescence, as in the case of tandem- conjugates.



Warranty

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