Anti-TCR Vβ3 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.
Anti-TCR Vβ3-VioBright FITC	9 µg in 300 µL	130-109-895
Anti-TCR Vβ3-VioBright FITC	30 µg in 1 mL	130-109-853
Anti-TCR Vβ3-PE	9 µg in 300 µL	130-109-892
Anti-TCR Vβ3-PE	30 µg in 1 mL	130-109-850
Anti-TCR Vβ3-APC	9 µg in 300 µL	130-109-893
Anti-TCR Vβ3-APC	30 µg in 1 mL	130-109-851
Anti-TCR Vβ3-PE-Vio770	9 µg in 300 µL	130-109-894
Anti-TCR Vβ3-PE-Vio770	30 µg in 1 mL	130-109-852
Anti-TCR Vβ3-APC-Vio770	30 µg in 1 mL	130-111-752
Anti-TCR Vβ3-Biotin	9 µg in 300 µL	130-109-891
Anti-TCR Vβ3-Biotin	30 µg in 1 mL	130-109-849

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	TCR Vβ3
Clone	REA646
Isotype	recombinant human IgG1
Isotype control	REA Control antibodies
Alternative names of antigen	TCR Vb3, Gm2426, Tcrb-V16
Molecular mass of antigen [kDa]	11
Distribution of antigen	T cells
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 REA646 recognizes the mouse V beta 3 T cell receptor (TCR Vβ3). The T cell receptor is responsible for recognizing antigens bound to major histocompatibility complex (MHC) molecules. It is a disulfide-linked membrane-anchored heterodimeric glycoprotein normally consisting of the highly

variable α and β chains expressed as part of a complex with the invariant CD3 chain molecules. The α and β TCR chains are composed of constant and variable regions, each encoded by distinct gene segments. TCR Vβ3 is a variant of the TCR β chain and is expressed on T cells having the a, b, and c haplotype of the TCR β gene complex.
Additional information: Clone REA646 displays negligible binding to Fc receptors.

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) 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.

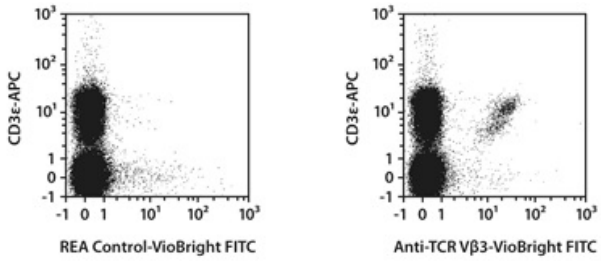
Protocol for cell surface staining

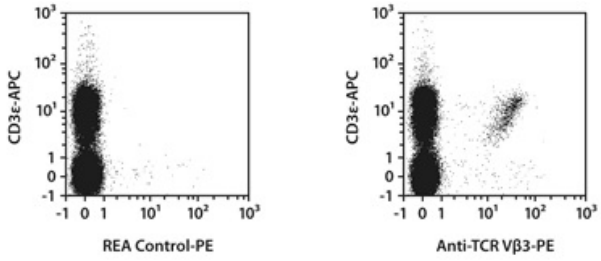
- The recommended antibody dilution for labeling of cells and subsequent analysis by flow cytometry is 1:10 for up to 10^6 cells/50 µL of buffer.
- Volumes given below are for up to 10^6 nucleated cells. When working with fewer than 10^6 cells, use the same volumes as indicated. When working with higher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

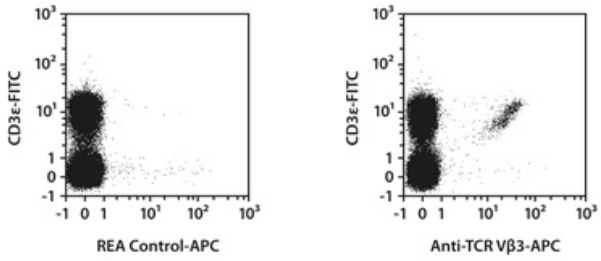
1. Determine cell number.
2. Centrifuge cell suspension at 300×g for 10 minutes. Aspirate supernatant completely.
3. Resuspend up to 10^6 nucleated cells per 45 µL of buffer.
4. Add 5 µL of the antibody.
5. Mix well and incubate for 10 minutes in the dark in the refrigerator (2−8 °C).
   Note: Higher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

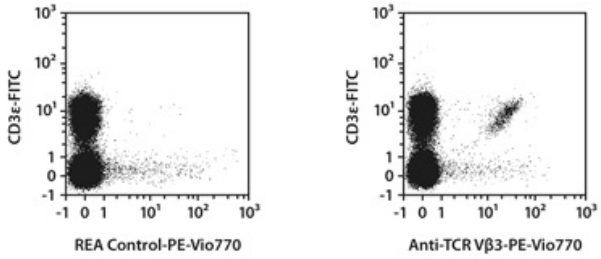
Examples of immunofluorescent staining

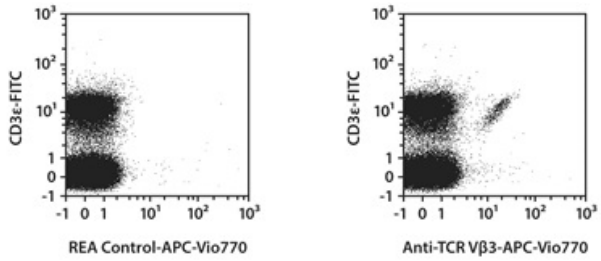
Splenocytes from C57BL/6 mice were stained with Anti-TCR Vβ3 antibodies or with the corresponding REA Control antibodies (left image) as well as with CD3ε antibodies. Flow cytometry was performed using the MACSQuant® Analyzer. Cell debris and dead cells were excluded from the analysis based on scatter signals and propidium iodide fluorescence or 4',6-diamidino-2-phenylindole (DAPI) fluorescence, as in the case of tandem conjugates.

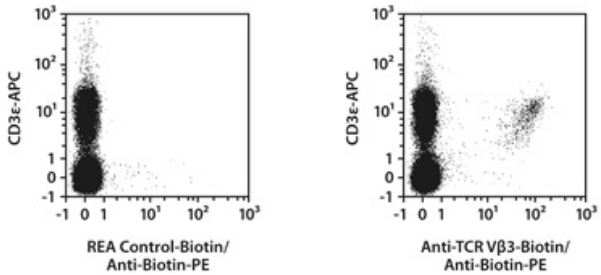

CD3ε-APC
REA Control-VioBright FITC
CD3ε-APC
Anti-TCR Vβ3-VioBright FITC


CD3ε-APC
REA Control-PE
CD3ε-APC
Anti-TCR Vβ3-PE


CD3ε-FITC
REA Control-APC
CD3ε-FITC
Anti-TCR Vβ3-APC


CD3ε-FITC
REA Control-PE-Vio770
CD3ε-FITC
Anti-TCR Vβ3-PE-Vio770


CD3ε-FITC
REA Control-APC-Vio770
CD3ε-FITC
Anti-TCR Vβ3-APC-Vio770


CD3ε-APC
REA Control-Biotin/
Anti-Biotin-PE
CD3ε-APC
Anti-TCR Vβ3-Biotin/
Anti-Biotin-PE

References

1. **Fairchild, S. *et al.*** (1992) Tcrb-V3^{+} T-cell deletion and a new mouse mammary tumor provirus, Mtv-44. Immunogenetics 36(3): 189–194.
2. **Tomonari, K. *et al.*** (1992) Tcrb-V3^{+} T-cell deletion and a mouse mammary tumor provirus, Mtv-27. Immunogenetics 36(5): 302–305.
3. **Lee, J. S. *et al.*** (2006) Antigen-specific expansion of TCR Vbeta3^{+} CD4^{+} T cells in the early stage of collagen-induced arthritis and its arthritogenic role in DBA/1J mice. J. Clin. Immunol. 26(3): 204–212.

Warranty

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