

Anti-TCR V β 5 antibodies, human

For research use only

One test corresponds to labeling of up to 10^6 cells in a total volume of 100 μ L

Product	Content	Order no.
Anti-TCR V β 5-PE	for 30 tests	130-115-091
Anti-TCR V β 5-PE	for 100 tests	130-115-049
Anti-TCR V β 5-APC	for 30 tests	130-115-092
Anti-TCR V β 5-APC	for 100 tests	130-115-050
Anti-TCR V β 5-PE-Vio770	for 30 tests	130-115-093
Anti-TCR V β 5-PE-Vio770	for 100 tests	130-115-051
Anti-TCR V β 5-APC-Vio770	for 30 tests	130-115-094
Anti-TCR V β 5-APC-Vio770	for 100 tests	130-115-052
Anti-TCR V β 5-Biotin	for 30 tests	130-115-089
Anti-TCR V β 5-Biotin	for 100 tests	130-115-047

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 V β 5
Clone	REA910
Isotype	recombinant human IgG1
Isotype control	REA Control (S) antibodies
Alternative names of antigen	TCR V β 5, TCR V β 5
Molecular mass of antigen [kDa]	10
Cross-reactivity	Pigtailed Macaque
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 REA910 recognizes the human variable β 5 subunit of the $\alpha\beta$ T cell receptor (TCR V β 5). The TCR is a heterodimeric glycoprotein associated with the CD3 antigen. The α and β TCR chains are composed of constant and variable regions, each encoded by distinct gene segments. TCR V β 5 is a

variant of the TCR β chain. $\alpha\beta$ T cells express a diverse $\alpha\beta$ TCR repertoire that specifically co-recognizes self or foreign antigen bound to antigen-presenting molecules, which thereby leads to T cell-mediated immunity. For example, the TCR can directly bind to peptide fragments, riboflavin precursors, and lipid antigens that are presented by major histocompatibility complex (MHC) molecules, MR1 and CD1, respectively. In each case, the antigen sits within the antigen-binding cleft, whereupon the TCR recognizes a composite surface formed by the antigen-presenting molecule and surface-exposed regions of the antigen itself. This co-recognition paradigm is a central tenet of $\alpha\beta$ T cell-mediated immunity and underpins MHC restriction.

Additional information: Clone REA910 displays negligible binding to Fc receptors.

Reagent requirements

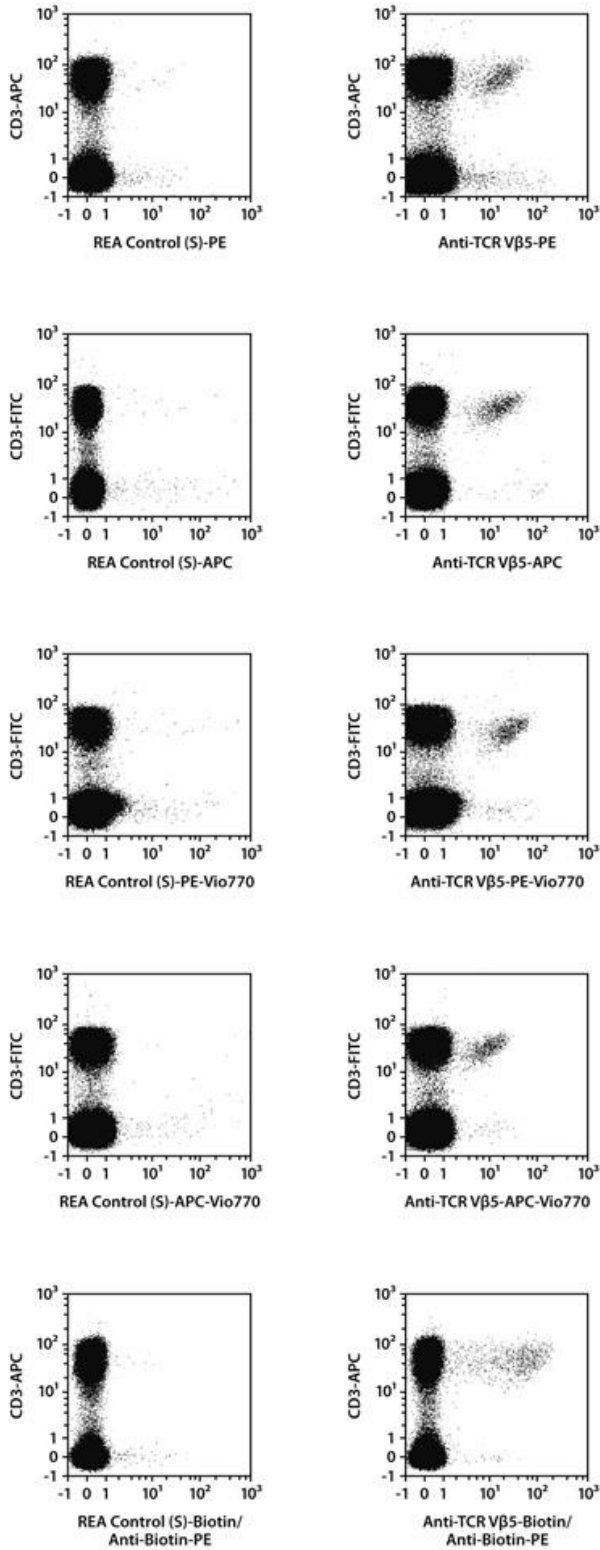
- Buffer: Prepare a solution containing phosphate-buffered saline (PBS), pH 7.2, 0.5% bovine serum albumin (BSA), and 2 mM EDTA by diluting MACS[®] BSA Stock Solution (# 130-091-376) 1:20 with autoMACS[®] Rinsing Solution (# 130-091-222). Keep buffer cold (2–8 °C).
Note: EDTA can be replaced by other supplements such as anticoagulant citrate dextrose formula-A (ACD-A) or citrate phosphate dextrose (CPD). Buffers or media containing Ca²⁺ or Mg²⁺ 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 1:50 for up to 10⁶ cells/100 μ L.
 - 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.
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 98 μ L of buffer.
 4. Add 2 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 buffer and stain with fluorochrome-conjugated anti-biotin antibody according to the manufacturer's recommendations.
 8. Resuspend cell pellet in a suitable amount of buffer for analysis by flow cytometry or fluorescence microscopy.

Examples of immunofluorescent staining

Human peripheral blood mononuclear cells (PBMCs) were stained with Anti-TCR V β 5 antibodies or with the corresponding REA Control (S) antibodies (left images) as well as with CD3 antibodies. Flow cytometry was performed using the MACSQuant[®] Analyzer. The Tandem Signal Enhancer has been used to increase binding specificity of tandem-dye-conjugated antibodies. 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.



References

1. Wei, S. *et al.* (1994) The extent of the human germline T-cell receptor V beta gene segment repertoire. *Immunogenetics* 40(1): 27–36.
2. Killestein, J. *et al.* (2002) Antibody-mediated suppression of Vbeta5.2/5.3⁺ T cells in multiple sclerosis: results from an MRI-monitored phase II clinical trial. *Ann. Neurol.* 51(4): 467–474.
3. Joedicke, J. J. *et al.* (2014) Activated CD8⁺ T cells induce expansion of Vβ5⁺ regulatory T cells via TNFR2 signaling. *J. Immunol.* 193(6): 2952–2960.

Warranty

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