

# Anti-TCR Vβ21.3 antibodies, human

# For research use only

One test corresponds to labeling of up to  $10^6$  cells in a total volume of  $100~\mu L$ 

Product	Content	Order no.
Anti-TCR Vβ21.3-Biotin	for 30 tests	130-114-878
Anti-TCR Vβ21.3-FITC	for 30 tests	130-114-879
Anti-TCR Vβ21.3-FITC	for 100 tests	130-114-837
Anti-TCR Vβ21.3-PE	for 30 tests	130-114-880
Anti-TCR Vβ21.3-PE	for 100 tests	130-114-838
Anti-TCR Vβ21.3-APC	for 30 tests	130-114-881
Anti-TCR Vβ21.3-APC	for 100 tests	130-114-839
Anti-TCR Vβ21.3-Biotin	for 100 tests	130-114-836

# **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 $\beta$ 21.3

Clone REA894

Isotyperecombinant human IgG1Isotype controlREA Control (S) antibodies

Alternative names of antigen TCR Vbeta 21.3, TCR V beta 21.3, TCR Vb21.3

Entrez Gene ID 28581

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 REA894 recognizes the human  $\beta$  21.3 subunit of the  $\alpha\beta$  T cell receptor (TCR V $\beta$ 21.3). The TCR is a disulfide-linked membrane-anchored heterodimeric protein associated with the CD3 antigen. The  $\alpha$  and  $\beta$  TCR chains are composed of constant and variable regions, each encoded by distinct gene segments. TCR V $\beta$ 21.3 is a variant of the TCR  $\beta$  chain.  $\alpha\beta$  T cells express a spanerse  $\alpha\beta$  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.

#### **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<sup>2+</sup> or Mg<sup>2+</sup> 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.

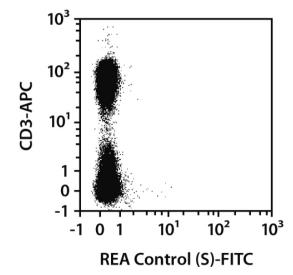
### **Protocol for cell surface staining**

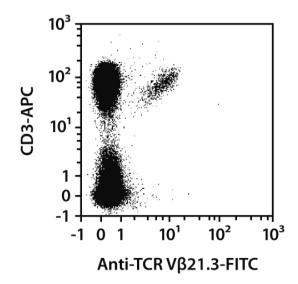
- $^{\circ}$  The recommended antibody dilution for labeling of cells and subsequent analysis by flow cytometry is 1:50 for up to  $10^{\circ}$  cells/100  $\mu$ L.
- $^{\bullet}$  Volumes given below are for up to  $10^{^{\circ}}$  nucleated cells. When working with fewer than  $10^{^{\circ}}$  cells, 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×g for 10 minutes. Aspirate supernatant completely.
- 3. Resuspend up to  $10^{\circ}$  nucleated cells per 98 µL of buffer.
- 4. Add 2  $\mu$ L of the antibody.
- 5. Mix well and incubate for 10 minutes in the dark in the refrigerator (2–8 °C).

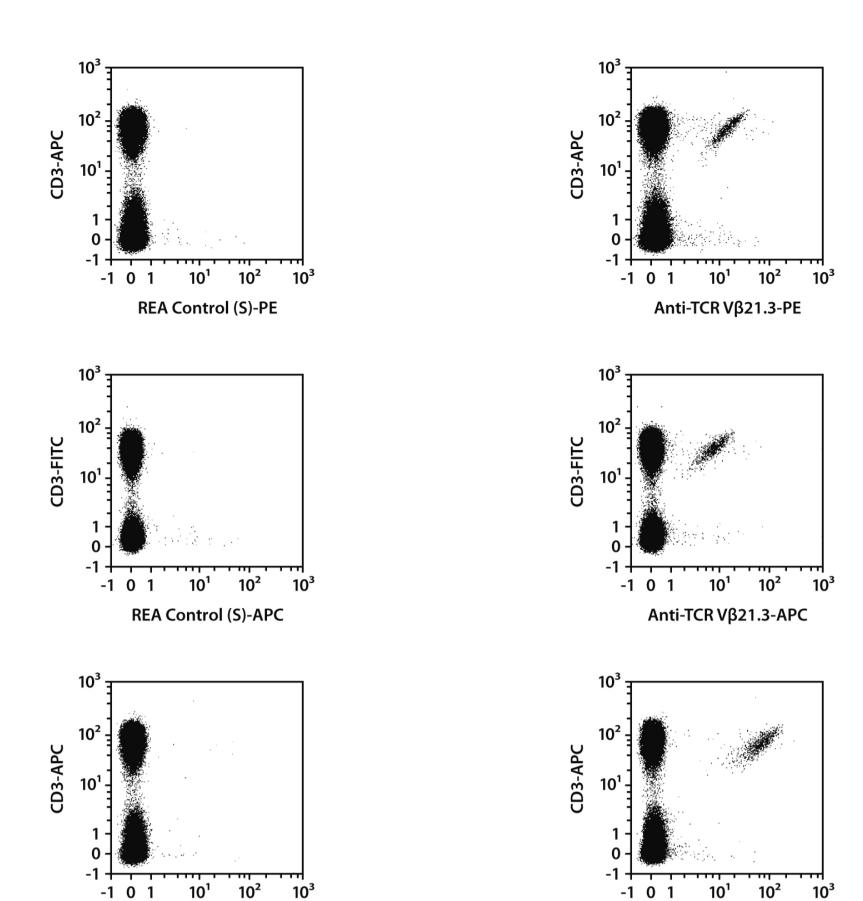
  Note: Higher temperatures and/or longer incubation times may lead to non-specific cell labeling. Working on ice requires increased incubation times.
- 6. Wash cells by adding 1-2 mL of buffer and centrifuge at  $300\times g$  for 10 minutes. Aspirate supernatant completely.
- 7. (Optional) If biotinylated antibody was used, resuspend the cell pellet in buffer and stain with fluorochrome-conjugated antibiotin 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 cells (PBMCs) were stained with Anti-TCR Vβ21.3 antibodies or with the corresponding REA Control (S) antibodies (left image) as well as with CD3 antibodies. Flow cytometry was performed using the MACSQuant<sub>®</sub>Analyzer. Cell debris and dead cells were excluded from the analysis based on scatter signals and propidium iodide fluorescence.







# Warranty

REA Control (S)-Biotin/

**Anti-Biotin-PE** 

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Anti-TCR Vβ21.3-Biotin/ Anti-Biotin-PE

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