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1. Description

Components	1 mL monoclonal Anti-TCR-V δ 2 antibodies, human conjugated to various dyes.	
	FITC	130-095-798
	PE	130-095-796
	APC	130-095-803
	Biotin	130-095-795
Clone	123R3 (isotype: mouse IgG1).	
Capacity	100 tests or up to 10 ⁹ total cells.	
Product format	Antibodies are supplied in buffer containing stabilizer and 0.05% sodium azide.	
Storage	Store protected from light at 2–8 °C. Do not freeze. The expiration date is indicated on the vial label.	

1.1 Background information

The Anti-TCR-V δ 2 antibody (clone 123R3) reacts with the V γ 9V δ 2 TCR chain on human V δ 2 T cells.

V δ 2 T lymphocytes are the major γ/δ T cell subset in humans. They recognize phosphoantigens, certain tumor cells, and cells treated with aminobisphosphonates, that are not seen by the T cell receptor of conventional α/β T cells. Activated V δ 2 T lymphocytes display strong cytotoxicity against various tumor cells and produce various cytokines, such as TNF- α and IFN- γ .

1.2 Applications

- Identification and enumeration of V δ 2⁺ T cells by flow cytometry.

1.3 Recommended antibody dilution

The recommended antibody dilution for all Anti-TCR-V δ 2 conjugates is **1:11 for up to 10⁷ cells/100 μ L** of buffer for labeling of cells and analysis by flow cytometry.

The antibody is suited for staining of formaldehyde-fixed cells.

For optimal results, cells must be stained after fixation with formaldehyde.

1.4 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). BSA can be replaced by other proteins such as human serum albumin, human serum, or fetal bovine serum (FBS). Buffers or media containing Ca²⁺ or Mg²⁺ are not recommended for use.
- (Optional) FcR Blocking Reagent, human (# 130-059-901) to avoid Fc receptor-mediated antibody labeling.
- (Optional) Anti-Biotin antibodies conjugated to, e.g., PE (# 130-090-756) as secondary antibody reagent in combination with Anti-TCR-V δ 2-Biotin.
- (Optional) Anti-FITC MicroBeads (# 130-048-701), Anti-PE MicroBeads (# 130-048-801), Anti-APC MicroBeads (# 130-090-855), or Anti-Biotin MicroBeads (# 130-090-485) for subsequent indirect magnetic labeling.
- (Optional) CD3-FITC (# 130-080-401), CD3-PE (# 130-091-374), or CD3-APC (# 130-091-373). For more information about antibodies refer to www.miltenyibiotec.com/antibodies.
- (Optional) Mouse IgG1 isotype control antibodies conjugated to, e.g., PE (# 130-092-212). For more information about isotype control antibodies refer to www.miltenyibiotec.com.
- (Optional) Propidium Iodide Solution (# 130-093-233) or 7-AAD 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.

2. General protocol for immunofluorescent staining

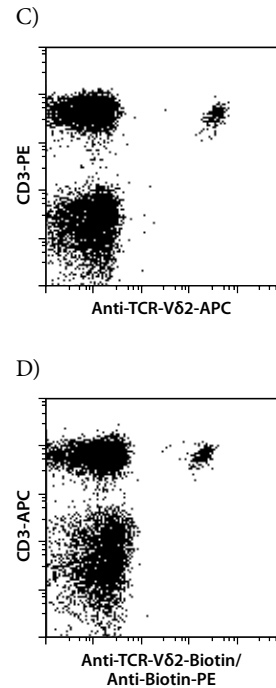
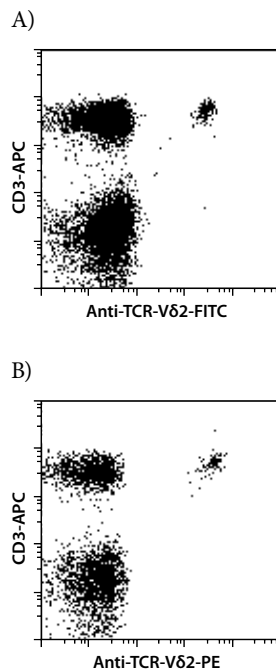
▲ Volumes given below are for up to 10⁷ nucleated cells. When working with fewer than 10⁷ cells, use the same volumes as indicated. When working with higher cell numbers, scale up all reagent volumes and total volumes accordingly (e.g. for 2×10⁷ nucleated cells, use twice the volume of all indicated 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 10⁷ nucleated cells per 100 μ L of buffer.
4. Add 10 μ L of the Anti-TCR-V δ 2 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 Anti-TCR-Vδ2-Biotin was used, resuspend the cell pellet in 100 µL of buffer, add 10 µL of 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.

3. Examples of immunofluorescent staining with Anti-TCR-Vδ2 antibodies

Human peripheral blood mononuclear cells (PBMCs) were stained with Anti-TCR-Vδ2 antibodies conjugated to FITC (A), PE (B), or APC (C) as well as with CD3-PE (# 130-091-374) or CD3-APC (# 130-091-373) and analyzed by flow cytometry using the MACSQuant® Analyzer. Cells labeled with Anti-TCR-Vδ2-Biotin (D) were stained with Anti-Biotin-PE (# 130-090-756) as well as CD3-APC. Cell debris and dead cells were excluded from the analysis based on scatter signals and propidium iodide fluorescence.



All protocols and data sheets are available at www.miltenyibiotec.com.

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.

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