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### 1. Description

<b>Components</b>	1 mL CD101 antibodies, human conjugated to various dyes.
	PE 130-095-225
	APC 130-095-132
	Biotin 130-095-221
<b>Clone</b>	BB27 (isotype: mouse IgG1).
<b>Capacity</b>	100 tests or up to 10 <sup>9</sup> total cells.
<b>Product format</b>	Antibodies are supplied in buffer containing stabilizer and 0.05% sodium azide.
<b>Storage</b>	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 CD101 antigen, also known as V7 or IGSF2, is a 140kDa type I transmembrane glycoprotein of the Ig gene superfamily. CD101 is expressed on monocytes, granulocytes, and subsets of dendritic cells (DCs). In addition, CD101 is expressed on subpopulations of T cells, in particular on about 5–30% of FoxP3<sup>+</sup> regulatory T cells (Tregs) in peripheral blood.

The engagement of CD101 with the antibody BB27 has been shown to inhibit T cell activation by allogeneic DCs or Anti-CD3.<sup>1,2,3</sup>

In addition to its role on T cells, triggering CD101 on human cutaneous DCs has been reported to inhibit T cell proliferation via IL-10 production, suggesting that the CD101 molecule may be involved in tolerance induction.<sup>4</sup>

#### 1.2 Applications

- Identification and enumeration of CD101<sup>+</sup> cells by flow cytometry or fluorescence microscopy.

#### 1.3 Recommended antibody dilution

The recommended antibody dilution for all CD101 conjugates is **1:11 for up to 10<sup>7</sup> cells/100 µL** of buffer for labeling of cells and analysis by flow cytometry.

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

#### 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 Ca<sup>2+</sup> or Mg<sup>2+</sup> 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 CD101-Biotin.
- (Optional) 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) CD4 (VIT4)-FITC (# 130-092-358), CD4 (VIT4)-VioBlue® (# 130-094-153), CD127-FITC (# 130-094-888), CD25-APC (# 130-092-858), or CD25-PE (# 130-091-024). For more information about antibodies refer to [www.miltenyibiotec.com/antibodies](http://www.miltenyibiotec.com/antibodies).
- (Optional) Mouse IgG1 isotype control antibodies conjugated to, e.g., PE (# 130-092-212) for isotype control. For more information about isotype control antibodies refer to [www.miltenyibiotec.com](http://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.

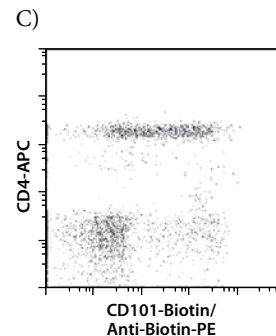
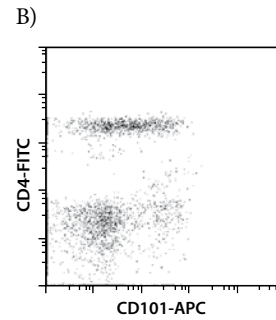
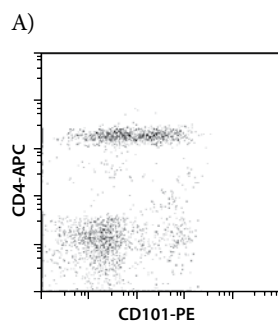
## 2. General protocol for immunofluorescent staining

▲ Volumes given below are for up to  $10^7$  nucleated cells. When working with fewer than  $10^7$  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  $2 \times 10^7$  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  $10^7$  nucleated cells per 100  $\mu$ L of buffer.
4. Add 10  $\mu$ L of the CD101 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 CD101-Biotin was used, resuspend the cell pellet in 100  $\mu$ L of buffer, add 10  $\mu$ L 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 CD101 antibodies

Human peripheral blood mononuclear cells (PBMCs) were stained with CD101 antibodies conjugated to PE (A) or APC (B) as well as with CD4 (VIT4)-FITC (# 130-092-358) and analyzed by flow cytometry using the MACSQuant® Analyzer. Cells labeled with CD101-Biotin (C) were stained with Anti-Biotin-PE (# 130-090-756) as well as CD4 (VIT4)-FITC. A lymphocyte gate was set and cell debris and dead cells were excluded from the analysis based on scatter signals and propidium iodide fluorescence.



## 4. References

1. Bagot, M. *et al.* (1997) CD101 is expressed by skin dendritic cells: role in T-lymphocyte activation. *Tissue Antigens* 50: 439–448.
2. Rivas, A. *et al.* (1995) V7, a novel leukocyte surface protein that participates in T cell activation. I. Tissue distribution and functional studies. *J. Immunol.* 154: 4423–4433.
3. Soares, L. R. *et al.* (1998) V7 (CD101) ligation inhibits TCR/CD3-induced IL-2 production by blocking  $Ca^{2+}$  flux and nuclear factor of activated T cell nuclear translocation. *J. Immunol.* 161: 209–217.
4. Bouloc, A. *et al.* (2000) Triggering CD101 molecule on human cutaneous dendritic cells inhibits T cell proliferation via IL-10 production. *Eur. J. Immunol.* 30: 3132–3139.

All protocols and data sheets are available at [www.miltenyibiotec.com](http://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.

### Warranty

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