

### Contents

1. Description
  - 1.1 Background information
  - 1.2 Applications
  - 1.3 Recommended antibody dilution
  - 1.4 Reagent requirements
2. General protocol for immunofluorescent staining
3. Examples of immunofluorescent staining with CD25 antibodies
4. References

### 1. Description

<b>Components</b>	1 mL monoclonal CD25 antibodies, human conjugated to various dyes.						
	<table border="0"> <tr> <td>PE</td> <td>130-091-024</td> </tr> <tr> <td>APC</td> <td>130-092-858</td> </tr> <tr> <td>Biotin</td> <td>130-091-235</td> </tr> </table>	PE	130-091-024	APC	130-092-858	Biotin	130-091-235
PE	130-091-024						
APC	130-092-858						
Biotin	130-091-235						
<b>Clone</b>	4E3 (isotype: mouse IgG2b).						
<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.						

Cross-reactivity: The CD25 antibody has been reported to react with a subset of peripheral blood mononuclear cells (PBMCs) from rhesus monkey (*Macaca mulatta*).

#### 1.1 Background information

CD25, the low affinity interleukin-2 receptor alpha chain (IL-2R $\alpha$ ), is expressed in the early phase (CD4<sup>+</sup>CD8<sup>-</sup>) of thymic T cell development as well as on activated T and B cells and at a lower level on activated monocytes. A subpopulation of CD4<sup>+</sup>CD25<sup>+</sup> T cells is discussed to act as regulatory T cells upon activation through their T cell receptor.<sup>1</sup> Regulatory CD4<sup>+</sup>CD25<sup>+</sup> T cells seem to suppress harmful immunological reactions to self or foreign antigens. In transfer experiments it was shown that CD4<sup>+</sup>CD25<sup>+</sup> T cells can prevent autoimmune reactions and maintain or reconstitute tolerance.<sup>2,3</sup>

The clone 4E3 recognizes epitope B of the antigen.

### 1.2 Applications

- Identification and enumeration of CD4<sup>+</sup>CD25<sup>+</sup> regulatory T cells obtained from PBMCs by flow cytometry or fluorescence microscopy. CD4<sup>+</sup>CD25<sup>+</sup> regulatory T cells can be isolated using the CD4<sup>+</sup>CD25<sup>+</sup> Regulatory T Cell Isolation Kit (# 130-091-301).
- Identification and enumeration of activated B cells by flow cytometry or fluorescence microscopy.
- Evaluation of MACS® Separations by flow cytometry or fluorescence microscopy. CD25<sup>+</sup> cells can be isolated by using CD25 MicroBeads II, human (# 130-092-983).

### 1.3 Recommended antibody dilution

The recommended antibody dilution for all CD25 conjugates is **1:11 for up to 10<sup>7</sup> cells/100  $\mu$ L** of buffer for labeling of cells and analysis by flow cytometry.

For CD25 MicroBead-labeled cells the dilution is **1:6 for up to 10<sup>7</sup> cells/100  $\mu$ L** of buffer.

### 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) Anti-Biotin antibodies conjugated to, e.g., PE (# 130-090-756) as secondary antibody reagent in combination with CD25-Biotin.
- (Optional) Mouse IgG2b isotype control antibodies conjugated to, e.g., APC (# 130-092-217). 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.

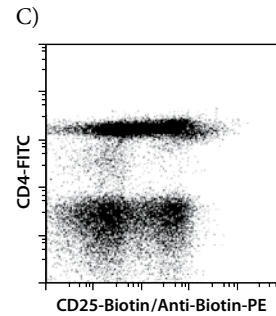
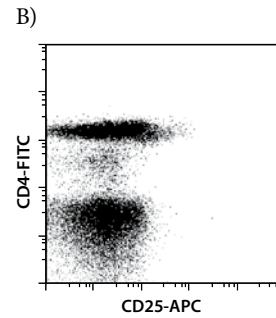
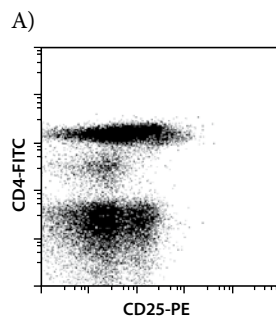
## 2. General protocol for immunofluorescent staining

▲ 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 CD25 antibody.  
▲ **Note:** Refer to section 1.3 for exceptions.
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 CD25-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 CD25 antibodies

Human PBMCs were stained with CD25 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 CD25-Biotin (C) were stained with Anti-Biotin-PE (# 130-090-756). Cell debris and dead cells were excluded from the analysis based on scatter signals and propidium iodide fluorescence.



## 4. References

1. Sakaguchi, S. *et al.* (1995) Immunologic self-tolerance maintained by activated T cells expressing IL-2 receptor alpha-chains (CD25). Breakdown of a single mechanism of self-tolerance causes various autoimmune diseases. *J. Immunol.* 155: 1151–1164.
2. Shevach, E. M. (2001) Certified professionals: CD4<sup>+</sup>CD25<sup>+</sup> suppressor T cells. *J. Exp. Med.* 193: F41–F46.
3. Maloy, K. J. and Powrie, F. (2001) Regulatory T cells in the control of immune pathology. *Nature Immunol.* 2: 816–822.

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

The products sold hereunder are warranted only to be free from defects in workmanship and material at the time of delivery to the customer. Miltenyi Biotec GmbH makes no warranty or representation, either expressed or implied, with respect to the fitness of a product for a particular purpose. There are no warranties, expressed or implied, which extend beyond the technical specifications of the products. Miltenyi Biotec GmbH's liability is limited to either replacement of the products or refund of the purchase price. Miltenyi Biotec GmbH is not liable for any property damage, personal injury or economic loss caused by the product.

autoMACS, MACS, and MACSQuant are registered trademarks of Miltenyi Biotec GmbH.

Copyright © 2010 Miltenyi Biotec GmbH. All rights reserved.