Miltenyi Biotec

Anti-FoxP3 antibodies
human and mouse

Anti-FoxP3-PE	130-093-014
Anti-FoxP3-APC	130-093-013

Contents

1. Description

Components	1 mL Anti-FoxP3 antibodies, human and mouse: monoclonal Anti-FoxP3 antibodies conjugated to R-phycoerythrin (PE) or allophycocyanin (APC).
Clone	3G3 (isotype: mouse IgG1).
Capacity	100 tests or up to 10^8 total cells.
Product format	Antibodies are supplied in a solution 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

FoxP3, also known as FORKHEAD BOX P3, SCURFIN, and JM2, is a member of the forkhead/winged-helix family of transcriptional regulators. It is expressed predominantly in regulatory T cells (Tregs) and is a major regulator of Treg cell development and function.[1,2,3] Mutations in the FoxP3 gene have been linked to the autoimmune manifestations observed in the Scurfy mouse and humans with immunedysregulation, polyendocrinopathy, enteropathy X-linked (IPEX) syndrome.[4,5] Studies in mice have shown that FoxP3-deficient animals lack Treg cells, whereas overexpression of the FoxP3 protein leads to profound immune suppression.[3] Clone 3G3 detects human as well as murine FoxP3.[6]

1.2 Applications

- Identification and enumeration of FoxP3$^+$ cells by flow cytometry or fluorescence microscopy.
- Analysis of Treg cells separated using MACS® Technology by flow cytometry or fluorescence microscopy. Human CD4$^+$CD25$^+$ Tregs can be isolated using the CD4$^+$CD25$^+$ Regulatory T Cell Isolation Kit, human (# 130-091-301). Mouse or non-human primate CD4$^+$CD25$^+$ Tregs can be isolated using either the CD4$^+$CD25$^+$ Regulatory T Cell Isolation Kit, mouse (# 130-091-041) or the CD4$^+$CD25$^+$ Regulatory T Cell Isolation Kit, non-human primate (# 130-092-984).

1.3 Recommended antibody dilution

For antibody labeling of human, mouse, or non-human primate cells.

Anti-FoxP3 conjugate	PE	APC
Flow cytometry[a] - Formaldehyde-fixed cells	1:11	1:11

a) Given antibody dilutions are for a cell concentration of up to 10^6 cells/100 µL of buffer.

- Cross-reactivity: The Anti-FoxP3 antibody is tested to react with mouse, rhesus monkey (*Macaca mulatta*), and cynomolgus (*Macaca fascicularis*) 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 calf serum. Buffers or media containing Ca^{2+} or Mg^{2+} are not recommended for use.
- FoxP3 Staining Buffer Set (# 130-093-142). The FoxP3 Buffer Set has been optimized for use with clone 3G3.

  ▲ **Note:** Use of the FoxP3 Staining Buffer Set is critical for optimal results. Always prepare solutions freshly and according to the data sheet supplied with the kit.

  ▲ **Caution:** Items within the FoxP3 Staining Buffer Set contain formaldehyde (EU Hazard Classification: Xn harmful; R40/20/21/22-43).
- CD4-FITC, human (# 130-092-358), CD25-PE, human (# 130-091-024), CD25-APC, human (# 130-092-858), CD25-PE, mouse (# 130-091-013), or CD4-FITC, mouse (# 130-091-608).
- FcR Blocking Reagent, human (# 130-059-901) or FcR Blocking Reagent, mouse (# 130-092-575) to avoid Fc receptor–mediated antibody labeling.

2. General protocol for immunofluorescent staining

▲ Volumes for fluorescent labeling given below are for **up to 10^6** nucleated cells. When working with fewer than 10^6 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 2×10^6 nucleated cells, use twice the volume of all indicated reagent volumes and total volumes).

2.1 Surface staining of Treg cells with CD4 and CD25 antibodies

1. Determine cell number.
2. Centrifuge cell suspension at 300×g for 10 minutes. Aspirate supernatant completely.
3. Resuspend up to 10^6 nucleated cells per 90 µL of buffer.
4. Add 10 µL of CD4-FITC and 10 µL of the CD25 antibody.
5. Mix well and incubate for 10 minutes in the dark in the refrigerator (2–8 °C).

   ▲ **Note:** Working on ice requires increased incubation times. Higher temperatures and/or longer incubation times may lead to non-specific cell labeling.

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6. Wash cells by adding 1–2 mL of buffer per 10^6 cells and centrifuge at 300×g for 5 minutes at 4 °C. Aspirate supernatant completely.
7. Proceed immediately to 2.2 Intracellular staining with the Anti-FoxP3 antibody.

2.2 Intracellular staining with the Anti-FoxP3 antibody

▲ For optimal intracellular staining, the FoxP3 Staining Buffer Set (# 130-093-142) must be used. Always prepare reagents freshly as recommended in the data sheet.

▲Working on ice requires increased incubation times. Higher temperatures and/or longer incubation times may lead to non-specific cell labeling.

1. Determine cell number.
2. Centrifuge cell suspension at 300×g for 10 minutes. Aspirate supernatant completely.
3. Resuspend up to 10^6 nucleated cells in 1 mL of cold, freshly prepared Fixation/Permeabilization Solution.
4. Mix well and incubate for 30 minutes in the dark in the refrigerator (2–8 °C).
5. Wash cells by adding 1–2 mL of cold buffer per 10^6 cells and centrifuge at 300×g for 5 minutes at 4 °C. Aspirate supernatant completely.
6. Wash cells by adding 1–2 mL of cold 1× Permeabilization Buffer per 10^6 cells and centrifuge at 300×g for 5 minutes at 4 °C. Aspirate supernatant completely.
7. Resuspend up to 10^6 nucleated cells in 80 µL of cold 1× Permeabilization Buffer.
8. Add 20 µL of FcR Blocking Reagent.

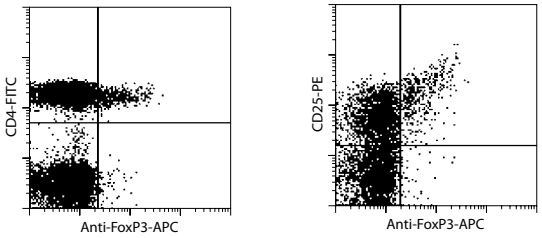
   ▲ **Note:** When working with mouse cells, use FcR Blocking Reagent, mouse.
9. Mix well and incubate for 5 minutes in the refrigerator (2–8 °C).
10. Add 10 µL of the Anti-FoxP3 antibody.
11. Mix well and incubate for 30 minutes in the dark in the refrigerator (2–8 °C).
12. Wash cells by adding 1–2 mL of cold 1× Permeabilization Buffer per 10^6 cells and centrifuge at 300×g for 5 minutes at 4 °C. Aspirate supernatant completely.
13. Resuspend cell pellet in a suitable amount of buffer for analysis by flow cytometry or fluorescence microscopy.

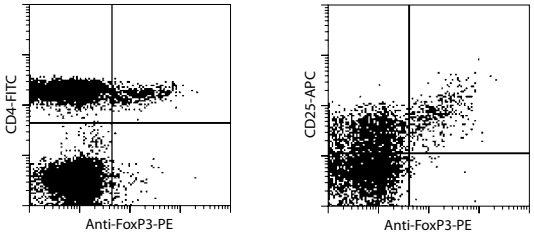
    ▲ **Note:** Due to fixation and permeabilization, cells are smaller than viable cells. Thus, FSC/SSC settings of the flow cytometer might need to be adjusted.

3. Examples of immunofluorescent staining with Anti-FoxP3 antibodies

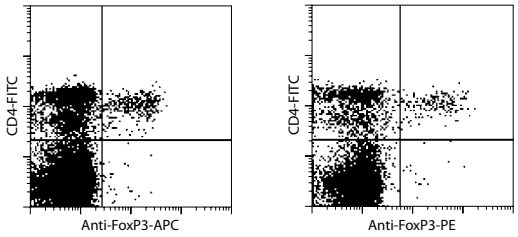
Human peripheral blood mononuclear cells (PBMCs) (a), mouse splenocytes (b), or rhesus monkey PBMCs (c) were stained with CD4-FITC and CD25-PE or CD25-APC, respectively. Cells were fixed, permeabilized, and stained with Anti-FoxP3-PE or Anti-FoxP3-APC. Cells were analyzed by flow cytometry. Autofluorescent cell debris was excluded in an FL-2 versus FL-3 dot plot.

(a) Human PBMCs stained with Anti-FoxP3-APC or Anti-FoxP3-PE.

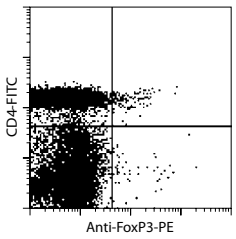




(b) Mouse spleen cells stained with Anti-FoxP3-APC or Anti-FoxP3-PE.



(c) Rhesus monkey PBMCs stained with Anti-FoxP3-PE.



4. References

1. Hori, S. *et al.* (2003) Control of regulatory T cell development by the transcription factor Foxp3. Science 299: 1057–1061.
2. Walker, M. R. *et al.* (2003) Induction of FoxP3 and acquisition of T regulatory activity by stimulated human $CD4^+CD25^-$ T cells. J. Clin. Invest. 112: 1437–1443.
3. Ziegler, S. F. (2006) FoxP3: Of Mice and Men. Annu. Rev. Immunol. 24: 209–26.
4. 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.
5. Lundsgaard, D. *et al.* (2005) *In vivo* control of diabetogenic T-cells by regulatory $CD4^+CD25^+$ T-cells expressing Foxp3. Diabetes 54: 306–310.
6. Gavin, M. A. *et al.* (2006) Single-cell analysis of normal and FOXP3-mutant human T cells: FOXP3 expression without regulatory T cell development. P.N.A.S. 103: 6659–6664.

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.

Warranty

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