Anti-FoxP3 antibodies, mouse

For research use only

30 µg equal 100 tests, 150 µg equal 500 tests. One test corresponds to labeling of 10 cells.

Product	Content	Order no.
Anti-FoxP3-Vio515	30 µg in 200 µL	130-111-681
Anti-FoxP3-PE	30 µg in 200 µL	130-111-678
Anti-FoxP3-PE	150 µg in 1 mL	130-111-600
Anti-FoxP3-APC	30 µg in 200 µL	130-111-679
Anti-FoxP3-APC	150 µg in 1 mL	130-111-601
Anti-FoxP3-Vio515	150 µg in 1 mL	130-111-603
Anti-FoxP3-Vio667	30 µg in 200 µL	130-111-682
Anti-FoxP3-Vio667	150 µg in 1 mL	130-111-604

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	FoxP3
Clone	REA788
Isotype	recombinant human IgG1
Isotype control	REA Control antibodies
Alternative names of antigen	AIID, DIETER, IPEX, JM2, PIDX, XPID
Entrez Gene ID	50943
Molecular mass of antigen [kDa]	47
Distribution of antigen	T cells
Product format	Reagents are supplied in buffer containing stabilizer and 0.05% sodium azide.
Fixation	The antibody is suited for staining of formaldehyde-fixed cells.
Storage	Store protected from light at 2–8 °C. Do not freeze.

Clone REA788 recognizes the forkhead/winged-helix transcriptional regulator FoxP3, also known as forkhead box P3, scurfin, or JM2. FoxP3 is expressed pre-dominantly in regulatory T cells (Tregs) and is a major marker and functional regulator of Treg cell development and function. Mutations in the FoxP3 gene are linked to the autoimmune manifestations observed in the Scurfy mouse and humans with immune dysregulation, polyendocrinopathy, enteropathy, X-linked (IPEX) syndrome. Studies in mice have shown that FoxP3-deficient animals lack Treg cells, whereas overexpression of the FoxP3 protein leads to profound immune suppression. Additional information: Clone REA788 displays negligible binding to Fc receptors.

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^{2+} or Mg^{2+} are not recommended for use.
- FoxP3 Staining Buffer Set (# 130-093-142) for cell fixation and permeabilization to analyze intranuclear proteins or transcription factors by flow cytometry.

Protocol for intracellular staining of cells

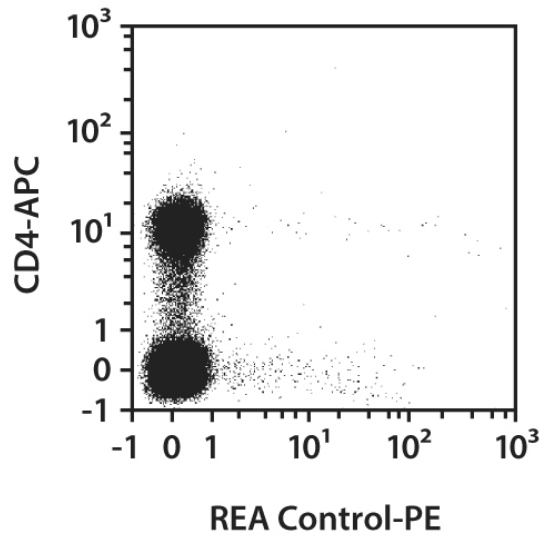
- The recommended antibody dilution for labeling of cells and subsequent analysis by flow cytometry is 1:50 for up to 10^6 cells/100 µL.
- Volumes given below are for up to 10^6 nucleated cells. When working with fewer than 10^6 cells, use the same volumes as indicated. When working with higher cell numbers, scale up all reagent volumes and total volumes accordingly.
- Always prepare Fixation/Permeabilization Solution freshly as described in the data sheet of the FoxP3 Staining Buffer Set (# 130-093-142).

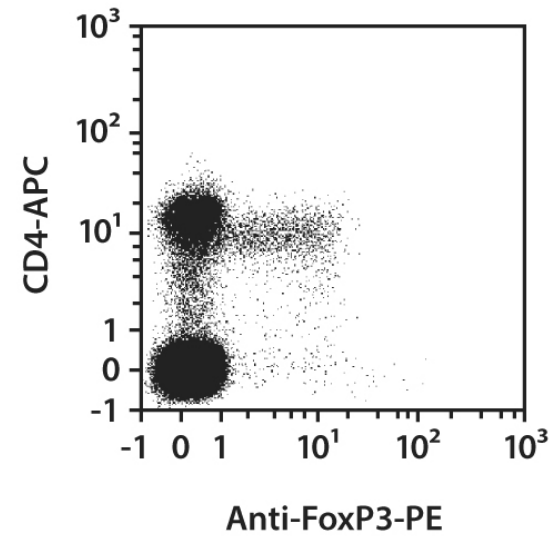
1. Wash up to 10^6 cells by adding 1–2 mL of buffer and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.
2. (Optional) Stain cell surface antigens with appropriate antibodies according to the manufacturer's recommendations. Then wash cells by adding 1–2 mL of buffer and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.
3. Resuspend up to 10^6 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 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 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 98 µL of cold 1× Permeabilization Buffer.
8. Add 2 µL of the antibody.
9. Mix well and incubate for 30 minutes in the dark in the refrigerator (2−8 °C).
10. Wash cells by adding 1 mL of cold 1× Permeabilization Buffer per 10^6 cells and centrifuge at 300×g for 5 minutes at 4 °C. Aspirate supernatant completely.
11. Resuspend cell pellet in a suitable amount of buffer for analysis by flow cytometry or fluorescence microscopy. Store cells at 2–8 °C in the dark until analysis. Mix well before flow cytometric acquisition.

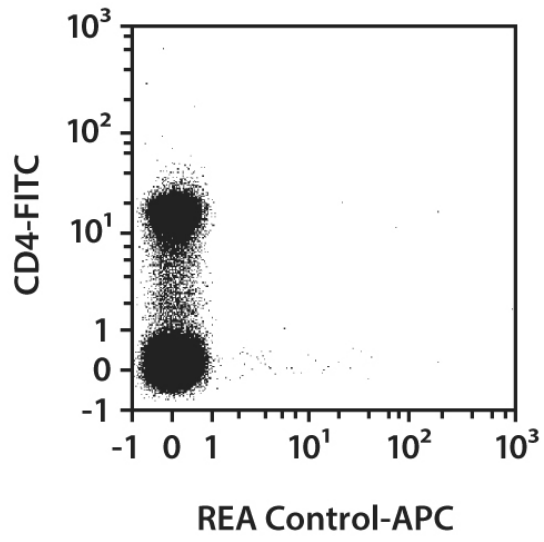
- Note: Do not use propidium iodide (PI) or 7-AAD staining.

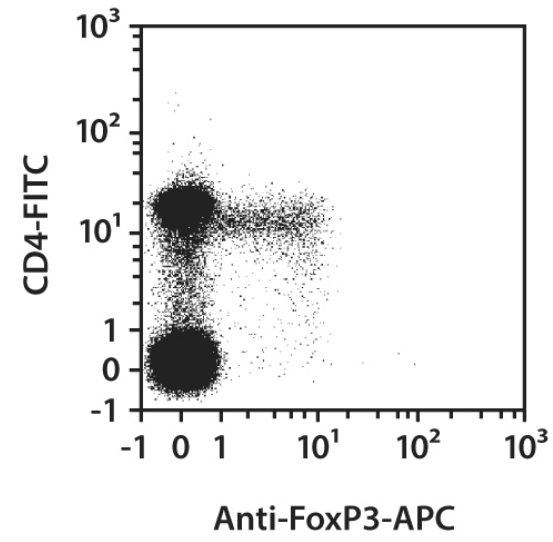
Examples of immunofluorescent staining

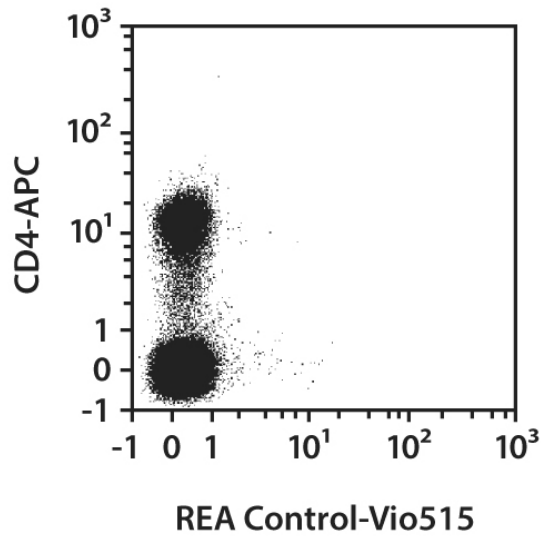
Splenocytes of C57BL/6 mice were fixed and permeabilized using the FoxP3 Staining Buffer Set. Cells were then stained with Anti-FoxP3 antibodies or with the corresponding REA Control antibodies (left image) as well as with CD4 antibodies. Flow cytometry was performed using the MACSQuant®Analyzer. Cell debris were excluded from the analysis based on scatter signals.

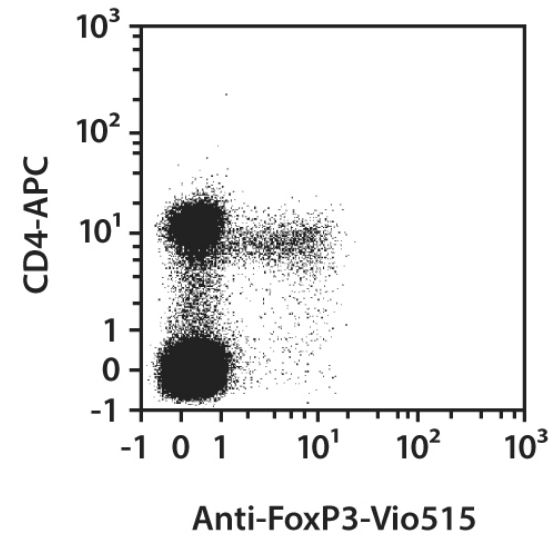


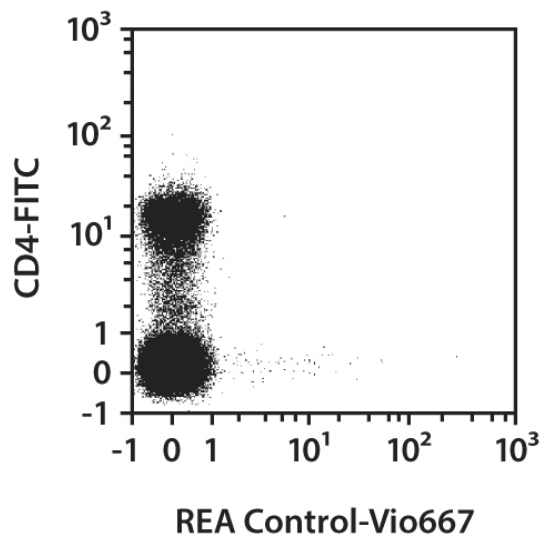


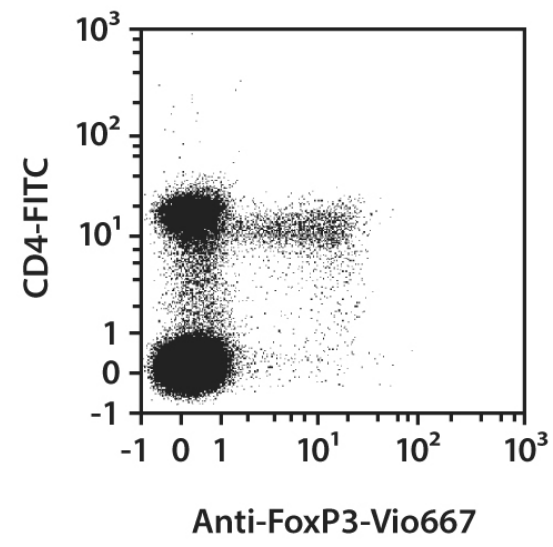












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