Human/Mouse FoxP3 Antibody

Recombinant Monoclonal Rabbit IgG Clone # 1054C
Catalog Number: MAB8214

DESCRIPTION

Species Reactivity	Human/Mouse
Specificity	Detects human FoxP3 in direct ELISAs. Detects human and mouse FoxP3 in flow cytometry.
Source	Recombinant Monoclonal Rabbit IgG Clone # 1054C
Purification	Protein A or G purified from cell culture supernatant
Immunogen	*E. coli*-derived recombinant human FoxP3 Met1-Leu71 Accession # Q9BZS1
Formulation	Lyophilized from a 0.2 μm filtered solution in PBS with Trehalose. See Certificate of Analysis for details. *Small pack size (-SP) is supplied either lyophilized or as a 0.2 μm filtered solution in PBS.

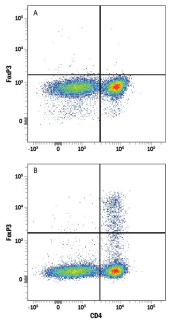
APPLICATIONS

Please Note: *Optimal dilutions should be determined by each laboratory for each application. General Protocols are available in the Technical Information section on our website.*

	Recommended Concentration	Sample
Dual RNAscope ISH-IHC Compatible	5-25 µg/mL	See Below
Flow Cytometry	0.25 µg/10^6 cells	See Below
Immunocytochemistry	8-25 µg/mL	See Below
Immunohistochemistry	5-25 µg/mL	See Below

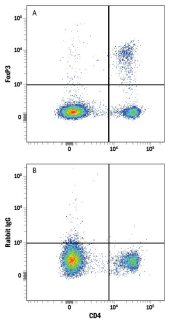
DATA

Flow Cytometry



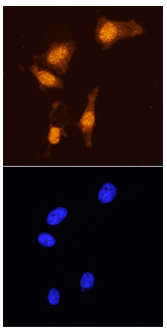
Detection of FoxP3 in Human PBMCs stimulated to induce Tregs by Flow Cytometry. Human peripheral blood mononuclear cells (PBMCs) either (A) untreated or (B) stimulated to induce Regulatory T Cells (Tregs) with Recombinant Human TGF-β1 (Catalog # 240-B) and Recombinant Human IL-2 (Catalog # 202-IL) for 2 days were stained with Rabbit Anti-Human/Mouse FoxP3 Monoclonal Antibody (Catalog # MAB8214) followed by Phycoerythrin-conjugated Anti-Rabbit IgG Secondary Antibody (Catalog # F0110) and Mouse Anti-Human CD4 APC-conjugated Monoclonal Antibody (Catalog # FAB3791A). Quadrant markers were set based on control antibody staining (Catalog # AB-105-C). To facilitate intracellular staining, cells were fixed and permeabilized with FlowX FoxP3 Fixation & Permeabilization Buffer Kit (Catalog # FC012).

Flow Cytometry



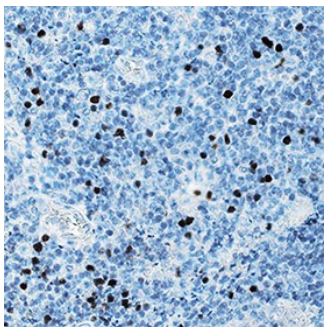
Detection of FoxP3 in Mouse Splenocytes by Flow Cytometry. Mouse splenocytes were stained with Rat Anti-Mouse CD4 APC-conjugated Monoclonal Antibody (Catalog # FAB554A) and either (A) Rabbit Anti-Human/Mouse FoxP3 Monoclonal Antibody (Catalog # MAB8214) or (B) Normal Rabbit IgG Control (Catalog # AB-105-C) followed by Phycoerythrin-conjugated Anti-Rabbit IgG Secondary Antibody (Catalog # F0110). To facilitate intracellular staining, cells were fixed and permeabilized with FlowX FoxP3 Fixation & Permeabilization Buffer Kit (Catalog # FC012).

Immunocytochemistry



FoxP3 in HeLa Human Cell Line. FoxP3 was detected in immersion fixed HeLa human cervical epithelial carcinoma cell line using Rabbit Anti-Human/Mouse FoxP3 Monoclonal Antibody (Catalog # MAB8214) at 8 µg/mL for 3 hours at room temperature. Cells were stained using the NorthernLights™ 557-conjugated Anti-Rabbit IgG Secondary Antibody (red, upper panel; Catalog # NL004) and counterstained with DAPI (blue, lower panel). Specific staining was localized to nuclei. View our protocol for Fluorescent ICC Staining of Cells on Coverslips.

Immunohistochemistry



FoxP3 in Human Tonsil. FoxP3 was detected in immersion fixed paraffin-embedded sections of human tonsil using Rabbit Anti-Human/Mouse FoxP3 Monoclonal Antibody (Catalog # MAB8214) at 15 µg/mL overnight at 4 °C. Tissue was stained using the Anti-Rabbit HRP-DAB Cell & Tissue Staining Kit (brown; Catalog # CTS005) and counterstained with hematoxylin (blue). Specific staining was localized to nuclei. View our protocol for Chromogenic IHC Staining of Paraffin-embedded Tissue Sections.

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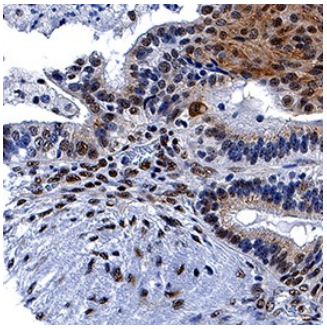
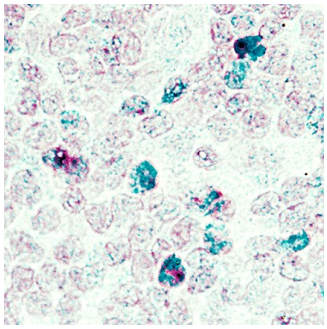
Immunohistochemistry



FoxP3 in Human Ovarian Cancer Tissue. FoxP3 was detected in immersion fixed paraffin-embedded sections of human ovarian cancer tissue using Rabbit Anti-Human/Mouse FoxP3 Monoclonal Antibody (Catalog # MAB8214) at 5 µg/mL for 1 hour at room temperature followed by incubation with the Anti-Rabbit IgG VisUCyte™ HRP Polymer Antibody (Catalog # VC003). Tissue was stained using DAB (brown) and counterstained with hematoxylin (blue). Specific staining was localized to nuclei. View our protocol for IHC Staining with VisUCyte HRP Polymer Detection Reagents.

Dual RNAscope ISH-IHC Compatible



FoxP3 in Human Tonsil Using Dual RNAscope® ISH and IHC. FoxP3 mRNA (red) and protein (green) was detected in formalin-fixed paraffin-embedded tissue sections of human tonsil probed with ACD RNAScope® Probe (Catalog # 418471) followed by immunohistochemistry using R&D Systems Rabbit Anti-Human/Mouse FoxP3 Monoclonal Antibody (Catalog# MAB8214) at 5ug/mL for 1 hour at room temperature followed by incubation with the Anti-Rabbit IgG VisUCyte HRP Polymer Antibody (R&D Systems, Catalog # VC003). Tissue was stained using ACD RNAscope® 2.5 HD Duplex Detection Reagents (Catalog # 322500).

PREPARATION AND STORAGE

Reconstitution	Reconstitute at 0.5 mg/mL in sterile PBS.
Shipping	The product is shipped at ambient temperature. Upon receipt, store it immediately at the temperature recommended below. *Small pack size (-SP) is shipped with polar packs. Upon receipt, store it immediately at -20 to -70 °C
Stability & Storage	**Use a manual defrost freezer and avoid repeated freeze-thaw cycles.** • 12 months from date of receipt, -20 to -70 °C as supplied. • 1 month, 2 to 8 °C under sterile conditions after reconstitution. • 6 months, -20 to -70 °C under sterile conditions after reconstitution.

BACKGROUND

Human FoxP3 is a 47 kDa member of the P subclass of the FOX (forkhead box) family of transcription factors. It contains a Leu-rich repeat, a C2H2 zinc finger region, and a C-terminal FKH (fork head), DNA-binding domain. Three isoforms for FoxP3 have been reported. All three isoforms share the sequence used as the immunogen. FoxP3 directly associates with NFAT and NFkB, suppressing their activity in $CD4^+$ T cells. In human, FoxP3 is found in $CD4^+$, $CD8^+$ and $CD4^+CD25^+$ T cells. Over the region used for immunization of the amino acid sequence, mouse FoxP3 is 83% to 88% identical to rat, human, canine, and bovine FoxP3.

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