Abstract

T regulatory cells (Tregs) play a key role in immune system suppression during autoimmunity and tumor development. Tregs are classified as CD4+CD25+FoxP3+, and exist as either natural Tregs (nTregs) derived from the thymus, or induced Tregs (iTregs) that develop from CD4+ T effector cells in the periphery. Reduction in Threg numbers leads to autoimmunity; this is clearly shown by the acutely mouse model in which FoxP3 has been deleted. Threg numbers are severely reduced, and mice succumb to autoimmunity early in life. In contrast, Tregs also play a deleterious role in cancer biology when Treg numbers are elevated, and the immune response is dampened allowing cancer cells to evade the immune system. Thus, the correct balance of Tregs is essential for maintaining immune homeostasis. FoxP3 has been widely used as a marker of Treg, and many antibodies are available on the market that distinguish the Treg population in human or mouse cells.

We developed a novel FoxP3 antibody and accompanying Fixation and Permeabilization buffer system that is comparable to current clones, but recognizes both mouse and human FoxP3 equally well, eliminating the need for separate antibodies. The mouse/human FoxP3 antibody was generated using our rabbit antibody technology, which allows for selecting high specificity, high affinity antibodies, lot to lot consistency and avoids mouse to human cross-reactivity.

Methods

Cell culture of mouse and human Tregs

PBMCs were isolated using a standard Ficoll gradient separation protocol. To generate PBMC Tregs, PBMCs were cultured in media containing 10% FBS, penicillin/streptomycin, Glutamine, sodium pyruvate, nonessential amino acids, and (β-mercaptoethanol) containing Recombinant Human IL-2 (20ng/mL; Catalog# 202-IL) and Human TGF-beta 1 (10ng/mL; Catalog# 100-B). C57Bl/6 or Balb/c splenocytes were isolated, lysed, and washed 2x with freshly made, cold 1x PBS. Resuspend cells in freshly made 1x Fixation Buffer using 0.5 mL/tube. Incubate at 4 °C for 30 minutes. Wash cells 2x with cold 1x PBS. Resuspend cells in FlowX FoxP3 Staining Buffer and analyze on a flow cytometer.

Development of a New Mouse/Human FoxP3 Antibody using Rabbit Antibody Technology

We developed a novel FoxP3 antibody and accompanying Fixation and Permeabilization buffer system that is comparable to current clones, but recognizes both mouse and human FoxP3 equally well, eliminating the need for separate antibodies. The mouse/human FoxP3 antibody was generated using our rabbit antibody technology, which allows for selecting high specificity, high affinity antibodies, lot to lot consistency and avoids mouse to human cross-reactivity. We developed a novel FoxP3 antibody and accompanying Fixation and Permeabilization buffer system that is comparable to current clones, but recognizes both mouse and human FoxP3 equally well, eliminating the need for separate antibodies. The mouse/human FoxP3 antibody was generated using our rabbit antibody technology, which allows for selecting high specificity, high affinity antibodies, lot to lot consistency and avoids mouse to human cross-reactivity. We developed a novel FoxP3 antibody and accompanying Fixation and Permeabilization buffer system that is comparable to current clones, but recognizes both mouse and human FoxP3 equally well, eliminating the need for separate antibodies. The mouse/human FoxP3 antibody was generated using our rabbit antibody technology, which allows for selecting high specificity, high affinity antibodies, lot to lot consistency and avoids mouse to human cross-reactivity. We developed a novel FoxP3 antibody and accompanying Fixation and Permeabilization buffer system that is comparable to current clones, but recognizes both mouse and human FoxP3 equally well, eliminating the need for separate antibodies. The mouse/human FoxP3 antibody was generated using our rabbit antibody technology, which allows for selecting high specificity, high affinity antibodies, lot to lot consistency and avoids mouse to human cross-reactivity.

Summary

• R&D Systems FoxP3 antibody is comparable and/or better than other FoxP3 antibodies on the market.
• One antibody can be used to detect FoxP3 in two different target species.
• A variety of antibodies work with the R&D Systems FlowX FoxP3 Fixation & Permeabilization Buffer Kit. Specifically, staining of intracellular cytokines using this kit looks virtually identical to the staining obtained with another vendor’s buffer set.
• Characteristics of R&D Systems Rabbit Anti-Mouse/Human FoxP3 Monoclonal Antibody:
  • High specificity
  • High affinity
  • Shows lot to lot consistency
  • Allows detection of both mouse and human nTregs and iTregs

Figure 1. Schematic of the FoxP3 immunensae. The rabbit monoclonal antibody for FoxP3 was generated using serum adsorbed 1:10 at the N-terminus of the FoxP3 protein. This monoclonal antibody cross-reacts in both mouse and human cells.

Figure 2. APC- and PE-conjugated FoxP3 Staining in Mouse and Human Tregs. To identify Tregs in human and mouse, cells were surface stained with the indicated fluorochrome-conjugated CD4 (α-CD4 PE and APC) and CD25/IL-2Rα (α-CD25/IL-2Rα PE) antibodies. The cells were then fixed and permeabilized using the FlowX™ FoxP3 Fixation & Permeabilization Buffer Kit (Catalog# FC012) and stained with R&D Systems Anti-Human FoxP3 APC or Anti-Mouse FoxP3 PE Antibody (Catalog# 1054-C and FoxP3 Clone #3, FoxP3 (1054-C) R&D Systems).

Figure 3. Alexa Fluor®488- and Alexa Fluor®700-conjugated FoxP3 Staining in Mouse and Human Tregs. To identify Tregs in human and mouse, cells were surface stained with the indicated fluorochrome-conjugated CD4 (α-CD4 PE and APC) and CD25/IL-2Rα (α-CD25/IL-2Rα PE) antibodies. The cells were then fixed and permeabilized using the FlowX™ FoxP3 Fixation & Permeabilization Buffer Kit (Catalog#FC012) and stained with R&D Systems Anti-Human FoxP3 Monoclonal Antibody (Catalog# IC8214) or with another vendor’s buffer set.

Figure 4. Comparison of R&D Systems APC-conjugated FoxP3 Antibody to Other FoxP3 Antibodies on the Mouse Using Human Tregs and Tgfs. PBMCs were stained as described in the Methods using A) R&D Systems FlowX FoxP3 Fixation & Permeabilization Buffer Kit (Catalog# FC012) or B) another vendor's buffer set. APC-conjugated Anti-Mouse/ Human FoxP3 Monoclonal Antibody (Catalog # 1054-C) was used as a control with FoxP3 to identify Tgfs. Both APC-titrations were stained with PE and reconstitute in 8 µL of 1x PBS for 4 hours with Monensin to induce 8.2 and 8.9% producers. The stimulated cells were fixed and permeabilized using the FlowX™ FoxP3 Fixation & Permeabilization Buffer Kit (Catalog# FC012) or another vendors buffer set. The cells were then stained with a fluorochrome-conjugated Anti-Mouse 4.2 Monoclonal Antibody (Catalog# IC8214) and an Anti-Mouse IL-2 Monoclonal Antibody (Catalog# 1195-M) to detect 8- and 8% production by flow cytometry.

Figure 5. R&D Systems FoxP3 Antibody Performance and Compatibility in Mouse Splenocytes. A) C57Bl/6 mouse splenocytes were stained as described in the Methods with R&D Systems APC-, PE- or Alexa Fluor-488-conjugated Anti-Mouse Human FoxP3 Monoclonal Antibody (Catalog# IC8214) with either R&D Systems FoxP3 Staining Buffer (A) or another vendor’s buffer set. The cells were then stained with a fluorochrome-conjugated Anti-Mouse CD25 PE Antibody (Catalog# IC485) to identify Tgfs. B) Mouse splenocytes were stained as described in the Methods using R&D Systems APC-conjugated Anti-Mouse/ Human FoxP3 Monoclonal Antibody (Catalog# 1054-C) to identify Tgfs and 8% production by flow cytometry.