

## Reagents Provided

### Allophycocyanin (APC)-conjugated goat polyclonal

**anti-human/mouse/rat FoxP3:** Supplied as 10 µg of antibody in 1 mL saline containing up to 0.5% BSA and 0.1% sodium azide.

**Isotype:** goat IgG

## Reagents Not Provided

**Flow Cytometry FoxP3 Staining Buffer (1X)** (Catalog # FC011)

*Required for optimal FoxP3 staining.*

**Flow Cytometry Staining Buffer (1X)** (Catalog # FC001) or other BSA-supplemented saline buffer.

## Storage

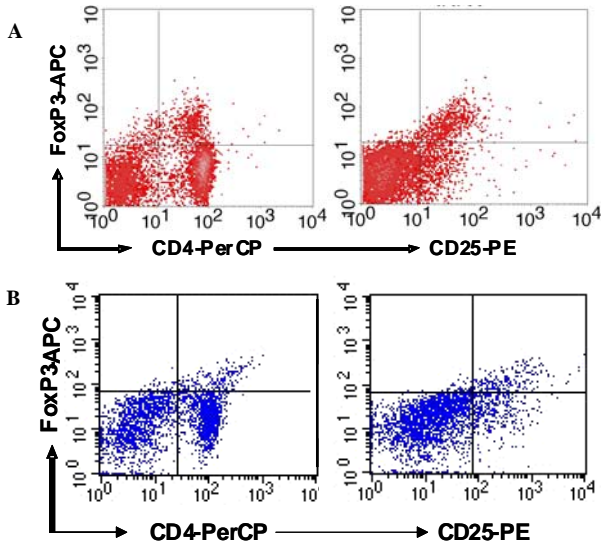
Reagents are stable for **twelve months** from the date of receipt when stored in the dark at 2° - 8° C.

## Intended Use

Designed to quantitatively determine the percentage of cells containing FoxP3 within a population and qualitatively determine the density of intracellular FoxP3 by flow cytometry.

## Product Description

Produced in goats immunized with purified, *E. coli*-derived, recombinant human Forkhead box P3 isoform 1 (rhFoxP3; aa 105 - 200; Accession # Q9BZS1). Human FoxP3 specific IgG was purified by human FoxP3 affinity chromatography. The purified antibody was then conjugated to APC fluorochrome. Intracellular expression of FoxP3 is determined by flow cytometry using 620 - 650 nm wavelength excitation and monitoring emitted fluorescence with a detector optimized to collect peak emissions at 660 - 670 nm.



A. Human PBMCs (top) were stained with APC-conjugated anti-human/mouse/rat FoxP3 (Catalog # IC3240A) and either PerCP-conjugated anti-human CD4 (# FAB3791C), or PE-conjugated anti-human CD25 (# FAB1020P). B. Mouse splenocytes (bottom) were stained with APC-conjugated anti-human/mouse/rat FoxP3 (# IC3240A) and either PerCP-conjugated anti-mouse CD4 (# FAB554C), or PE-conjugated anti-mouse CD25 (# FAB2438P).

FOR RESEARCH USE ONLY. NOT FOR USE IN HUMANS.

## Background Information

Human FOXP3 is a 47 kDa member of the P subclass of the FOX (forkhead box) family of transcription factors. It contains a leucine-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 NFκB, suppressing their activity in CD4<sup>+</sup> T cells. In human, FOXP3 is found in CD4<sup>+</sup>, CD8<sup>+</sup>, and CD4<sup>+</sup>CD25<sup>+</sup> 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.

## Flow Cytometry Validation

For intracellular staining, cells must first be fixed and permeabilized using R&D Systems **Flow Cytometry FoxP3 Staining Buffer (1X)** (Catalog # FC011). **This buffer must be used for optimal FoxP3 staining.** See buffer insert for detailed staining protocol. **Note: Different fix/perm buffers may change side-scatter/forward-scatter patterns of the stained cells, therefore, gating all live cells is recommended.**

1. Cells are harvested and washed twice in saline buffer.
2. Cell surface staining may be done at this point following the manufacturer's staining procedure.
3. Wash the cells in 1 mL of **Flow Cytometry FoxP3 Staining Buffer** (Catalog # FC011).
4. Decant buffer, then add 10 µL of conjugated antibody and incubate the cells for 1 hour at 2° - 8° C **in the dark**.
5. Wash the cells with **Flow Cytometry FoxP3 Staining Buffer**.
6. Resuspend the cells in saline buffer for final flow cytometric analysis. As a control for this analysis, cells in a separate tube should be treated with APC-labeled goat IgG antibody. This procedure may need to be modified, depending on the cell type and final utilization. Individual users may need to titrate to determine the optimal reagent amount for their specific use.

**Warning:** Contains sodium azide as a preservative - sodium azide may react with lead and copper plumbing to form explosive metal azides. Flush with large volumes of water during disposal.