R&D systems
a bio-techne brand

# FlowX™ Mouse Regulatory T Cell
## Multi-Color Flow Cytometry Kit

**Catalog Number:** FMC022

**Size:** 25 Tests

## PRODUCT DESCRIPTION

This kit contains three conjugated antibodies (and a rabbit IgG-PE isotype control) that can be used for the single-step staining of mouse regulatory T cells (1-9).

## MATERIALS PROVIDED & STORAGE

Store the unopened kit at 2-8 °C **in the dark.** Use within 6 months of receipt.

| | PART | CATALOG #/PART # | DESCRIPTION |
|---|---|---|---|
| Positive Markers | Human/Mouse FoxP3-PE Rabbit IgG; Clone 1054C | IC8214P | 250 µL of a phycoerythrin-conjugated antibody specific for human/mouse FoxP3. |
| | Mouse CD4-FITC Rat $IgG_{2B}$; Clone GK1.5 | FAB554F | 250 µL of a fluorescein-conjugated antibody specific for mouse CD4. |
| | Mouse IL-2 Rα/CD25-APC Rat $IgG_{2A}$; Clone 280406 | FAB2438A | 250 µL of an allophycocyanin-conjugated antibody specific for mouse IL-2 Rα/CD25. |
| Isotype Control | Rabbit IgG Control-PE | IC105P | 250 µL of a phycoerythrin-conjugated control specific for rabbit IgG. |
| Staining Buffers | FoxP3 Fixation Concentrate (4X) | 894065 | 8 mL of a formaldehyde solution. |
| | FoxP3 Fixation Diluent | 894068 | 25 mL of a buffered detergent. |
| | FoxP3 Permeabilization and Wash Buffer (10X) | 894356 | 25 mL of a buffered protein base with detergent and preservatives. **Note:** *May contain a precipitate but will not affect product performance.* |
| | Staining Buffer (1X) | 895068 | 50 mL of a 1X Staining Buffer |

## INTENDED USE

This product is designed for the flow cytometric analysis of mouse regulatory T cells using three fluorochrome-conjugated antibodies.

## STAINING PROTOCOL

1. Wash mouse splenocytes (1 x $10^6$ cells per sample) with 2 mL of Flow Cytometry Staining Buffer (R&D Systems, Catalog # FC001) or other BSA-containing buffer, by spinning at 300 x g for 5 minutes, using 5 mL flow cytometry tubes.
2. Fc-block cells with blocking IgG (1 µg IgG/$10^6$ cells) for 10 minutes at 2-8 °C.
3. Add 10 µL of CD4-FITC and 10 µL of CD25-APC antibodies or isotype controls (R&D Systems, Catalog # IC013F and IC006A).
4. Incubate the mixture for 30-45 minutes at 2-8 °C **in the dark**.
5. Wash the cells two times with **cold** 1X PBS. During the washes, make up fresh 1X FoxP3 Fixation Buffer by diluting FoxP3 Fixation Concentrate (4X) with FoxP3 Fixation Diluent (*i.e.* 100 µL FoxP3 Fixation Concentrate (4X) + 300 µL FoxP3 Fixation Diluent).
6. Resuspend the cells in fresh 1X FoxP3 Fixation Buffer using 0.5 mL/tube. Incubate at 2-8 °C for 30 minutes. During this incubation, make up 1X FoxP3 Permeabilization and Wash Buffer by diluting FoxP3 Permeabilization and Wash Buffer (10X) with distilled water (*i.e.* 100 µL FoxP3 Permeabilization and Wash Buffer (10X) + 900 µL di$H_2O$) and keep at 2-8 °C.
7. Wash two times with fresh, cold, 1X FoxP3 Permeabilization and Wash Buffer.
8. Add 10 µL of FoxP3 antibody or the rabbit IgG-PE isotype control included in this kit to the cells and incubate for 30 minutes at 2-8 °C.
9. Wash the cells one time with cold 1X FoxP3 Permeabilization and Wash Buffer.
10. Resuspend the cells in Flow Cytometry Staining Buffer and run on a flow cytometer.



bio-techne.com
info@bio-techne.com
techsupport@bio-techne.com

North America
TEL 800 343 7475

Europe • Middle East • Africa
TEL +44 (0)1235 529449

China
info.cn@bio-techne.com
TEL +86 (21) 52380373

Rest of World
bio-techne.com/find-us/distributors
TEL +1 612 379 2956

## DATA EXAMPLES

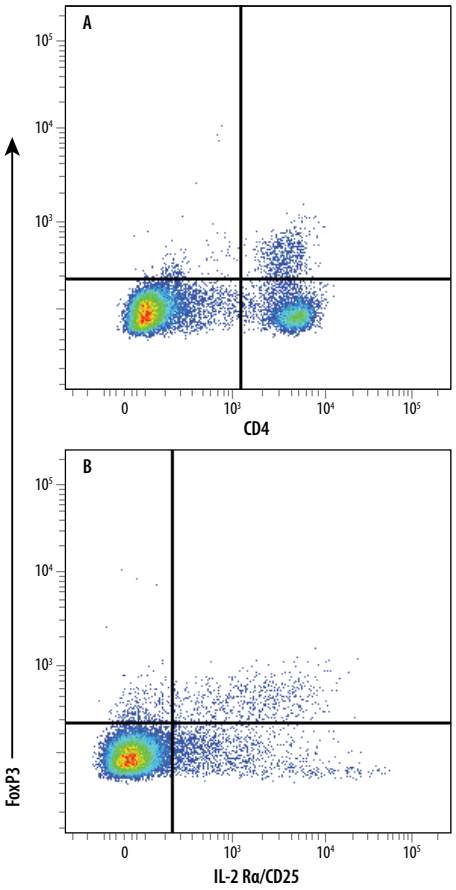


**Figure 1: Detection of FoxP3 in CD57/B6 Mouse Splenocytes Natural Tregs by Flow Cytometry.** CD57/B6 mouse splenocyte natural regulatory T cells (Tregs) were surface stained with **(A)** Rat Anti-Mouse CD4 Fluorescein-conjugated Monoclonal Antibody (R&D Systems, Catalog # FAB554F) and **(B)** Rat Anti-Mouse IL-2 Rα/CD25 APC-conjugated Monoclonal Antibody (R&D Systems, Catalog # FAB2438A), followed by intracellular staining using Rabbit Anti-Human/Mouse FoxP3 PE-conjugated Antigen Affinity-purified Monoclonal Antibody (R&D Systems, Catalog # IC8214P). To facilitate intracellular staining, cells were fixed and permeabilized with FlowX FoxP3 Fixation & Permeabilization Buffer Kit (R&D Systems, Catalog # FC012). Cells were gated on lymphocytes.

## PRECAUTIONS

FoxP3 Staining Buffer contains formaldehyde which is a suspected carcinogen. Avoid contact with skin, eyes, and mucous membranes, and avoid inhaling fumes. In case of contact, wash immediately with water and seek medical advice.

The Staining Buffers in this kit contain sodium azide which may react with lead and copper plumbing to form explosive metallic azides. Flush with large volumes of water during disposal.

## REFERENCES


1. Tang, Q. and J.A. Bluestone (2008) Nature Immunol. **9**:239.
2. Zheng, Y. and A.Y. Rudensky (2007) Nature Immunol. **8**:457.
3. Hori, S. *et al.* (2003) Science **299**:1057.
4. Kim, J.M. and A.Y. Rudensky (2006) Immunol. Rev. **212**:86.
5. Fontenot, J.D. *et al.* (2003) Nat. Immunol. **4**:330.
6. Khatri, R. *et al.* (2003) Nat. Immunol. **4**:337.
7. Bluestone, J.A. and A.K. Abbas (2003) Nat. Rev. Immunol. **3**:253.
8. Sakaguchi, S. (2004) Ann. Rev. Immunol. **22**:531.
9. Josefowicz, S.Z. and A.Y. Rudensky (2009) Immunity **30**:616.