R&D SYSTEMS
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FlowX™ Rat Regulatory T Cell
Multi-Color Flow Cytometry Kit

Catalog Number: FMC015B

Size: 25 Tests

PRODUCT DESCRIPTION

This kit contains three conjugated antibodies (and a mouse IgG_1 isotype control) that can be used for single-step staining of rat regulatory Treg cells (1-9).

MATERIALS PROVIDED & STORAGE

Store the unopened kit at 2-8 °C **in the dark.** Refer to the kit label for date of expiration.

PART	PART #	DESCRIPTION
Positive Markers	968435	125 µL of FoxP3 Alexa Fluor® 647 Mouse IgG_1 (Clone 376209)
	968436	250 µL of CD25/IL-2 Rα-PE Mouse IgG_1 (Clone 745520)
	967211	250 µL of CD4-Fluorescein Mouse IgG_{2A} (Clone OX-38)
Isotype Control	968440	250 µL of Mouse IgG_1 Alexa Fluor® 647 Mouse IgG_1 (Clone 11711)
FoxP3/Transcription Factor Fixation Concentrate (4X)	894065	8 mL of a formaldehyde solution.
FoxP3/Transcription Factor Fixation Diluent	894068	25 mL of a buffered detergent.
FoxP3/Transcription Factor Permeabilization & Wash Buffer (10X)	894356	25 mL of a buffered protein base with detergent and preservatives. *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 Treg cells using three fluorochrome-conjugated antibodies.

PRECAUTIONS

Some components in this kit contain 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.

Some components 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.
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4. Kim, J.M. and A.Y. Rudensky (2006) Immunol. Rev. **212**:86.
5. Fontenot, J.D. *et al.* (2003) Nat. Immunol. **4**:330.
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7. Bluestone, J.A. and A.K. Abbas (2003) Nat. Rev. Immunol. **3**:253.
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INTRACELLULAR STAINING PROTOCOL

1. Harvest cells and wash with 2.0 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 5.0 mL flow cytometry tubes.
2. Fc-block cells with blocking IgG (1.0 µg IgG/10^6 cells) for 10 minutes at 2-8 °C, if desired.
3. Surface stain the cells by adding 10 µL of CD4-Fluorescein and 10 µL of CD25-PC.
4. Incubate 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/Transcription Factor Fixation Buffer by diluting FoxP3/Transcription Factor Fixation Concentrate (4X) with FoxP3/Transcription Factor Fixation Diluent (*i.e.* 100 µL FoxP3/Transcription Factor Fixation Concentrate (4x) + 300 µL FoxP3/Transcription Factor Fixation Diluent).
6. Resuspend the cells in fresh 1X FoxP3/Transcription Factor Fixation Buffer using 0.5 mL/tube. Incubate at 2-8 °C for 30 minutes. During this incubation, make up 1X FoxP3/Transcription Factor Permeabilization and Wash Buffer by diluting FoxP3/Transcription Factor Permeabilization and Wash Buffer (10X) with distilled water (*i.e.* 100 µL FoxP3/Transcription Factor Permeabilization and Wash Buffer (10X) + 900 µL di$H_2$0) and keep at 2-8 °C.
7. Wash two times with fresh, cold, 1X FoxP3/Transcription Factor Permeabilization and Wash Buffer.
8. Add 5.0 µL of FoxP3-Alexa Fluor® 647 to the cells and incubate for 30 minutes at 2-8 °C.
9. Wash the cells one time with cold 1X FoxP3/Transcription Factor Permeabilization and Wash Buffer.
10. Resuspend the cells in Flow Cytometry Staining Buffer and run on a flow cytometer.

TECHNICAL HINTS

- Isotype controls may be used to set quadrant markers if desired: Mouse IgG_1-PE (R&D Systems®, Catalog # IC002P), Mouse IgG_1-Fluorescein (R&D Systems®, Catalog # IC002F), and Mouse IgG_1-Alexa Fluor® 647 (R&D Systems®, Catalog # IC002R, included in the kit).
- A live/dead fixable viability dye may be used to exclude dead cells from analysis.
- Doublet exclusion gating is recommended to gate specifically on single cells.

DATA EXAMPLES

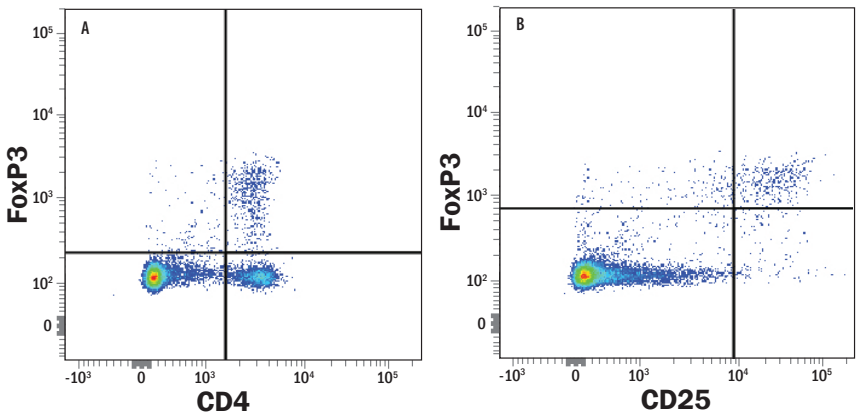

Figure 1: Rat splenocytes were harvested and stained with the indicated antibodies following the procedure. Live, single cells are shown in the dot plots (determined using a fixable viability dye and doublet exclusion). Dot plots show relative $CD4^+$, $CD25^+$, $FoxP3^+$ cells. Quadrant markers were set based on isotype controls.