R&D SYSTEMS
a bio-techne brand

Rat Regulatory T Cell
Multi-Color Flow Cytometry Kit

Catalog Number: FMC015
Size: 50 Tests

PRODUCT DESCRIPTION

This kit contains three conjugated antibodies (and a goat IgG-APC isotype control) that can be used for single-step staining of rat regulatory T 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	967138	500 µL of FoxP3-APC Goat IgG
	967210	500 µL of CD25-PE Goat IgG
	967211	500 µL of CD4-FITC Mouse IgG_{2A} (Clone OX-38)
Isotype Control	967140	500 µL of Goat IgG-APC
Staining Buffers	895068	50 mL of 1X Staining Buffer
	895947	120 mL of FoxP3 Staining Buffer

INTENDED USE

This product is designed for the single-step staining and flow cytometric analysis of rat regulatory T cells using three fluorochrome-conjugated antibodies.

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.
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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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STAINING PROTOCOL

1. Wash rat splenocytes (about 1 x 10^6 cells per sample) with 2 mL of PBS or Hanks' Balanced Salt Solution (HBSS), by spinning at 300 x g for 5 minutes, using 5 mL flow cytometry tubes.
2. Remove all remaining PBS or HBSS and resuspend the samples in 100 µL of Flow Cytometry Staining Buffer.
3. Add 10 µL of CD4-FITC and 10 µL of CD25-PE antibodies or isotype controls (R&D Systems®, Catalog # IC003F and IC108P).
4. Incubate the mixture for 30-45 minutes at room temperature **in the dark.**
5. Following the incubation, remove any excess antibody by washing the cells with 1 mL of Flow Cytometry FoxP3 Staining Buffer.
6. After decanting the Flow Cytometry FoxP3 Staining Buffer, add 10 µL of FoxP3-APC antibody or goat IgG-APC isotype control to the samples in the remaining small volume of buffer (about 100 µL).
7. Incubate the mixture for 1 hour at room temperature **in the dark.**
8. Following the incubation, remove any excess antibody by washing the cells with 1 mL of Flow Cytometry FoxP3 Staining Buffer.
9. The final cell pellet is resuspended in 200-400 µL of Flow Cytometry Staining Buffer for flow cytometric analysis.

   Note: *Different fixation/permeabilization buffers might change side-scatter/forward-scatter patterns of the stained cells. Therefore, gating all live cells is recommeneded.*

DATA EXAMPLES

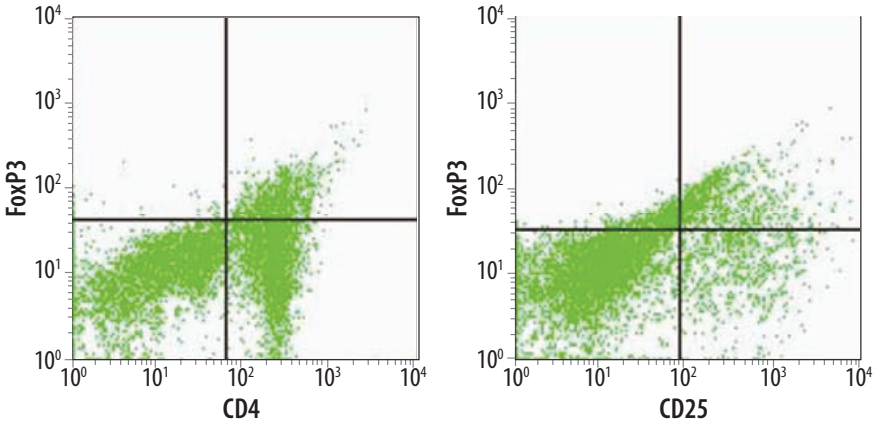

Figure 1: Rat splenocytes were harvested and stained with the indicated antibodies following the staining protocol outlined above. Dot plots show the relative $CD4^+$, $CD25^+$, and $FoxP3^+$ populations. Quadrants were set based on isotype controls.