R&D SYSTEMS
a bio-techne brand

Mouse Th2 Cell
Multi-Color Flow Cytometry Kit
Catalog Number: FMC012
Size: 25 Tests

PRODUCT DESCRIPTION
This kit contains four conjugated antibodies (and corresponding isotype controls) that can be used for single-step staining of mouse Th2 cells (1-5):

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

PART	PART #	DESCRIPTION
Positive Markers	967109	250 µL of STAT6-APC, Mouse IgG_{2A}; Clone 253906
	967111	250 µL of IL-5-PE, Rat IgG_1; Clone TRFK5
	967112	250 µL of IL-4 R-CFS, Goat IgG; Antibody-Purified Polyclonal Antibody
	967113	250 µL of CD4-PerCP, Rat IgG_{2B}; Clone GK1.5
Isotype Controls	967114	250 µL of Rat IgG_{2B}-PerCP Isotype Control
	965667	250 µL of Mouse IgG_{2A}-APC Isotype Control
	965947	250 µL of Rat IgG_1-PE Isotype Control
	965949	250 µL of Goat IgG-CFS Isotype Control
Fixation/Permeabilization Buffer	895029	30 mL of 1X Fixation/Permeabilization Buffer
Permeabilization/Wash Buffer	895030	2 bottles (30 mL/bottle) of 1X Permeabilization/Wash Buffer

INTENDED USE
This product is designed for the flow cytometric analysis of mouse PSCs using four fluorochrome-conjugated antibodies.

PRECAUTIONS
The fixation/Permeabilization Buffer provided in this kit 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.

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.

INTRACELLULAR STAINING PROTOCOL WITH SIMULTANEOUS FIXATION/PERMEABILIZATION
1. Harvest cells of interest and wash twice in PBS or Hanks' Balanced Salt Solution (HBSS).
2. Approximately 5 x 10^5 washed cells should be resuspended in 0.5 mL of Fixation/Permeabilization Buffer and incubated at 2-8 °C for 30 minutes. To maintain a single cell suspension, cells should be vortexed intermittently.
3. Centrifuge the cells and resuspend the pellet in 100-200 µL of the Permeabilization/Wash Buffer.
4. Add 10 µL of each antibody or each corresponding isotype control antibody to the cells.
5. Incubate the mixture for 30-45 minutes at 2-8 °C in the dark.
6. Following the incubation, remove any excess antibody by washing the cells in 2 mL of Permeabilization/Wash Buffer. Resuspend the final cell pellet in 200-400 µL of PBS for flow cytometric analysis.

   Notes: *Because saponin-mediated cell permeabilization is a reversible process, it is important to keep the cells in the presence of saponin during intracellular staining. Using multiple fluorochromes requires proper flow cytometric compensation to remove the spillover fluorescence from a particular probe to a certain channel (6).*

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DATA EXAMPLES

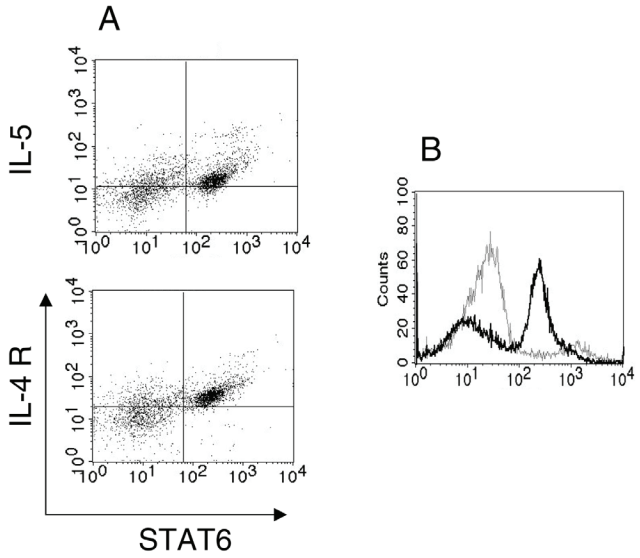

Figure 1: For Th2 activation, mouse splenocytes were cultured for 72 hours with recombinant mouse IL-4 (5 ng/mL; R&D Systems®, Catalog # 404-ML), anti-mouse IFN-γ (10 µg/mL; R&D Systems®, Catalog # AF-485-NA), anti-CD3ε (10 µg/mL; R&D Systems®, Catalog # MAB484), and anti-CD28 (10 µg/mL; R&D Systems®, Catalog # MAB4831) followed by a 3 hour re-stimulation with PMA/ionomycin. Cells were harvested and stained with the indicated antibodies following the procedure. Dot plots **(A)** show the relative IL-4 R^+, IL-5^+, and $STAT6^+$ populations from activated splenocytes on $CD4^+$-gated cells. Quadrants were set based on isotype controls. The histogram **(B)** shows the relative number of $STAT6^+$ cells in unstimulated (gray line) versus stimulated (black line) splenocytes.

REFERENCES

1. Chang, S. and T.M. Aune (2007) Nature Immunol. **8**:723.
2. Zhou, L. *et al.* (2009) Immunity **30**:646.
3. Ho, I.C. *et al.* (2009) Nat. Rev. Immunol. **9**:125.
4. Nakayama, T. and M. Yamashita (2008) Curr. Opin. Immunol. **20**:265.
5. Spilianakis, C.G. and R.A. Flavell (2004) Nat. Immunol. **5**:1017.
6. Bagwell, B. and E.G. Adams (1993) Ann. N.Y. Acad. Sci. **677**:167.