R&D SYSTEMS
a bio-techne brand

Mouse Th1 Cell
Multi-Color Flow Cytometry Kit
Catalog Number: FMC010
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 Th1 cells (1-5).

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	965711	250 µL of CD4-PE Rat IgG_{2B}; Clone GK1.5
	967096	250 µL of IL-12 Rβ2-APC Mouse IgG_1; Clone 305719
	965712	250 µL of IFN-γ-CFS Rat IgG_{2A}; Clone 37895
	965713	250 µL of T-bet-PerCP Mouse IgG_1; Clone 525803
Isotype Controls	967107	250 µL of Rat IgG_{2B}-PE Isotype Control
	965675	250 µL of Mouse IgG_1-APC Isotype Control
	965715	250 µL of Rat IgG_{2A}-CFS Isotype Control
	965669	250 µL of Mouse IgG_1-PerCP 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 Th1 cells 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 of this kit contain sodium azide 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. Resuspend approximately 5 x 10^5 washed cells in 0.5 mL of Fixation/Permeabilization Buffer and incubate at 2-8 °C for 30 minutes. The cells should be vortexed intermittently in order to maintain a single cell suspension.
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 add 10 µL of each corresponding isotype control antibody to the cells.
5. Incubate the mixture for 30-45 minutes at room temperature **in the dark**.
6. Following the incubation, remove any excess antibody by washing the cells in 2 mL of Permeabilization/Wash Buffer. The final cell pellet is resuspended 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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Bio-Techne is a trading name for R&D Systems

DATA EXAMPLES

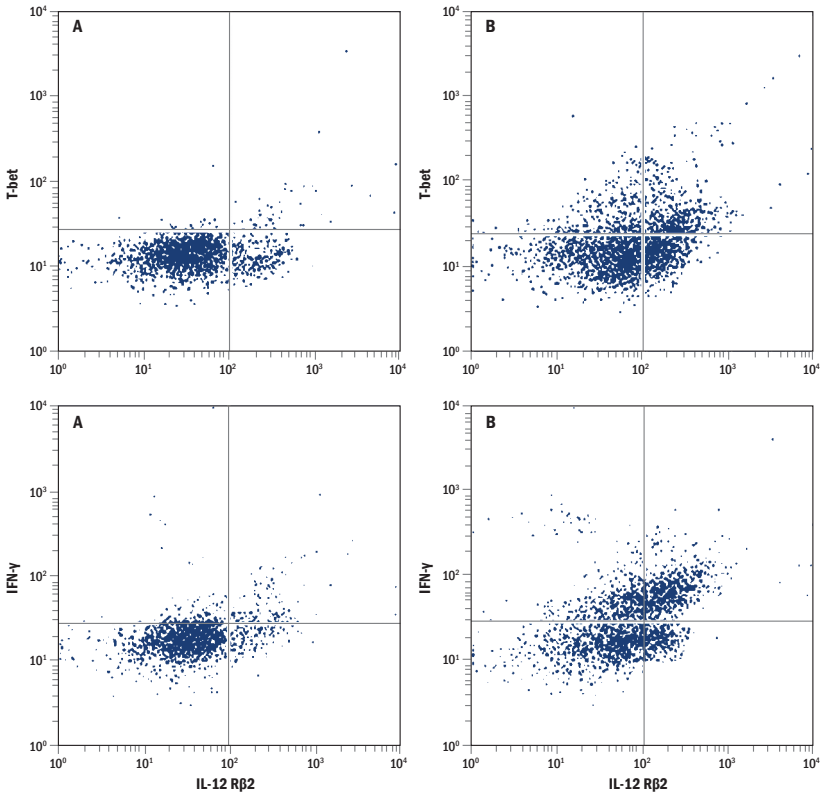

Figure 1: For Th1 activation, mouse splenocytes were cultured for 72 hours with 5 ng/mL of recombinant mouse IL-12 (R&D Systems®, Catalog # 419-ML), 10 µg/mL of anti-IL-4 (R&D Systems®, Catalog # AF-404-NA), 10 µg/mL of anti-CD3 (R&D Systems®, Catalog # MAB484), and 10 µg/mL of anti-CD28 (R&D Systems®, Catalog # MAB4831) followed by 3 hours of re-stimulation with PMA-ionomycin and 3 µM of monensin. Cells were harvested and stained with the indicated antibodies following the procedure. Dot plots show the relative T-bet$^+$, IFN-γ$^+$, and IL-12 Rβ2$^+$ populations from inactivated **(A)** and activated **(B)** CD4$^+$-gated splenocytes. Quadrants were set based on isotype controls.

REFERENCES

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