R&D SYSTEMS
a bio-techne brand

FlowX™ Human Th2 Cell

Multi-Color Flow Cytometry Kit

Catalog Number: FMC011B

Size: 25 Tests

PRODUCT DESCRIPTION

This kit contains five conjugated antibodies and buffers that can be used for staining of human Th2 cells (1-6).

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	968426	125 µL of CD4 Alexa Fluor® 700 Mouse IgG_{2A} (Clone 11830)
	968433	125 µL of IL-10 Alexa Fluor® 488 Rabbit IgG (Clone 2050B)
	968432	250 µL of CCR3-PE Rat IgG_{2A} (Clone 61828)
	968434	125 µL of GATA-3 Alexa Fluor® 594 Mouse IgG_{2B} (Clone 634919)
	968431	125 µL of Phospho-STAT6 Alexa Fluor® 647 Rabbit IgG (Clone 1248D)
Fixation/Permeabilization Buffer (1X)	895029	30 mL of 1X fixation buffer.
Staining Buffer (1X)	895068	50 mL of a 1X staining buffer.

OTHER MATERIALS REQUIRED

- Methanol (ice cold)

INTENDED USE

This product is designed for the flow cytometric analysis of Th2 cells using five 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. Chang, S. and T.M. Aune (2007) Nature Immunol. **8**:723.
2. Lee, G.R. *et al.* (2006) Immunity **24**:369.
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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

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

Bio-Techne is a trading name for R&D Systems

GENERATION OF HUMAN TH2 DIFFERENTIATED CD4$^+$ T CELLS

The following protocol may be used to differentiate human PBMCs to Th2 cells. Alternatively, the CellXVivo™ Human Th2 Cell Differentiation Kit (R&D Systems®, Catalog # CDK002), or other protocols may be used to generate Th2 cells.

1. Prepare cell culture flasks or plates containing immobilized anti-human CD3 and CD28 antibodies: Add 5.0 µg/mL of anti-human CD3ε antibody (R&D Systems®, Catalog # MAB100) and 2.0 µg/mL of anti-human CD28 antibody (R&D Systems®, Catalog # AF-342-PB) in PBS to the cell culture flask or plate and incubate at 4 °C overnight. The next day, wash two times with PBS.
2. Purify CD4$^+$ T cells from total human PBMCs using a cell selection protocol, such as MagCellect™ Human CD4$^+$ T cell Isolation Kit (R&D Systems®, Catalog # MAGH102).
3. Resuspend cells in complete RPMI media containing 10 ng/mL of recombinant human IL-4 (R&D Systems®, Catalog # 204-IL) and 20 µg/mL of anti-human IFN-γ antibody (R&D Systems®, Catalog # AF-285-NA) and incubate at 37 °C for 5 days. Media and soluble factors should be replenished every 2-3 days.
4. On day 5, stimulate the cells with 50 ng/mL PMA and 200 ng/mL Calcium Ionomycin for 1 hour, then add a secretion inhibitor such as Monensin (3.0 µM) for an additional 3 hours.

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 5.0 µL of CD4-Alexa Fluor® 700 and 10 µL of CCR3-PE.
4. Incubate for 30-45 minutes at 2-8 °C **in the dark**.
5. Wash the cells two times with 1X PBS. Resuspend the cells in Fixation/Permeabilization Buffer (1X) using 0.5 mL/tube. Incubate at room temperature for 10 minutes. Vortex cells intermittently in order to maintain a single cell suspension.
6. Following fixation, wash two times with PBS.
7. For permeabilization, add 1.0 mL ice-cold methanol to each tube and vortex. Incubate for 30 minutes at 2-8 °C.
8. Wash the cells two times in 1X PBS.
9. Add 5.0 µL of phospho-STAT6-Alexa Fluor® 647, 5.0 µL of IL-10 Alexa Fluor® 488, and 5.0 µL of GATA-3 Alexa Fluor® 594 to the cells and incubate for 30 minutes at 2-8 °C.
10. Wash the cells one time with cold 1X PBS.
11. 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_{2A}-Alexa Fluor® 700 (R&D Systems®, Catalog # IC003N), Rabbit IgG-Alexa Fluor® 488 (R&D Systems®, Catalog # IC1051G), Rat IgG_{2A}-PE (R&D Systems®, Catalog # IC006P), Mouse IgG_{2B} Alexa Fluor® 594 (R&D Systems®, Catalog # IC0041T) and Rabbit IgG Alexa Fluor® 647 (R&D Systems®, Catalog # IC1051R).
- 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

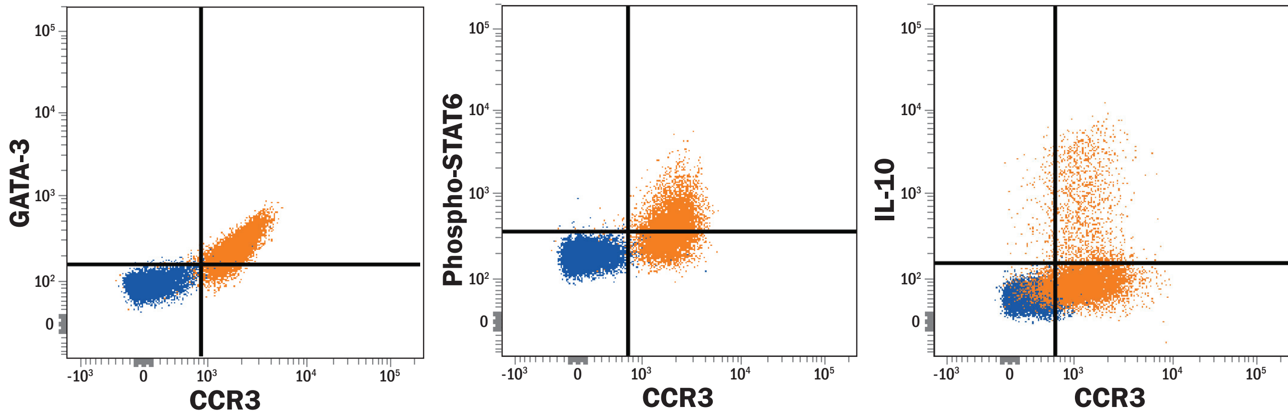

Figure 1: Th2 cells were generated using the protocol above. Cells were harvested and stained with the indicated antibodies following the procedure. Live, single, CD4+ cells are shown in the dot plots (determined using a fixable viability dye, doublet exclusion, and staining with anti-human CD4-Alexa Fluor® 700). Dot plots show relative IL-10+, CCR3+, GATA-3+, phospho-STAT6+ cells in CD4+ resting (blue dots, lower left quadrant) and Th2-differentiated (orange dots, right quadrants). Quadrant markers were set based on isotype controls.