**DATA EXAMPLES**

Differentiation of naive CD4+ T cells into Th2 cells is confirmed by intracellular staining for IL-4 (Figure 1) and secretion of IL-5 (Figure 2). The corresponding tests for IFN-γ (Th1 cell marker) and IL-17 (Th17 cell marker) are low/negative.

Figure 1: Intracellular Cytokine Staining of Differentiated Human Th2 Cells. Flow cytometry showing human peripheral blood naïve CD4+ T cells without (A, C) and with (B, D) a 13 day differentiation using reagents included in the Human Th2 Cell Differentiation Kit. On day 13 of differentiation, the cells were re-stimulated with mitogens and stained with Human IL-17, Human IFN-γ, and Human IL-4 Monoclonal Antibodies. Quadrants were set based on isotype-stained samples. All R&D Systems antibodies and corresponding catalog numbers used in this figure are shown below.

**SUGGESTED REAGENTS FOR FLOW CYTOMETRY**

<table>
<thead>
<tr>
<th>CATALOG #</th>
<th>DESCRIPTION</th>
</tr>
</thead>
<tbody>
<tr>
<td>IC285A, and IC604A</td>
<td>Human IFN-γ APC MAb (Clone 25723), Mouse IgG4, and Mouse IgG4-APC Isotype Control (Clone J31330)</td>
</tr>
<tr>
<td>IC286P, and IC602P</td>
<td>Human IL-4 Phycoerythrin MAb (Clone 1007), Mouse IgG and Mouse IgG-Phycoerythrin Isotype Control (Clone 11711)</td>
</tr>
<tr>
<td>IC317IC, and IC600C</td>
<td>Human IL-17 PerCP MAb (Clone 41802), Mouse IgG, and Mouse IgG-PerCP Isotype Control (Clone 11711)</td>
</tr>
<tr>
<td>FAB1979E, and IC602F</td>
<td>Human CD4 Fluorescein MAb (Clone 118310), Mouse IgG4, and Mouse IgG4-Fluorescein Isotype Control (Clone 20102)</td>
</tr>
<tr>
<td>FCX04</td>
<td>Flow Cytometry Fixation Buffer (1X)</td>
</tr>
<tr>
<td>FCX05</td>
<td>Flow Cytometry Permeabilization/Wash Buffer (1X)</td>
</tr>
</tbody>
</table>

**REFERENCES**


**HUMAN TH2 CELL DIFFERENTIATION KIT**

Catalog Number: CDK002

**BACKGROUND**

T helper type 2 (Th2) cells are a lineage of CD4+ effector T cells that provide host protection against intestinal helminths and extracellular bacteria in addition to support for B cell-dependent humoral responses. Pathological Th2 cell activity is a hallmark of allergic inflammation and asthma (1). Differentiation of CD4+ effector cells into the Th2 lineage is promoted by cytokines such as IL-4 in combination with either IL-2, IL-7, or TSLP (2, 3). Th2 cells secrete IL-4, IL-5, IL-9, IL-13, and IL-17E/IL-25. The CellXVivo Human Th2 Cell Differentiation Kit contains all necessary components to differentiate human naïve CD4+ T cells into Th2 polarized cells.

This package insert must be read in its entirety before using this product. For research use only. Not for use in diagnostic procedures.
**MATERIALS PROVIDED & STORAGE CONDITIONS**

Store the unopened kit at 2-8 °C. Do not use past kit expiration date.

**COMPONENTS** | PART # | # VIALS | **STORAGE OF OPENED/ RECONSTITUTED MATERIAL**
---|---|---|---
Mouse Anti-Human CD3 | 967538 | 1 vial | May be stored at 2-8 °C under sterile conditions for up to 30 days or at -20 °C to -70 °C in a manual defrost freezer for up to 3 months.*
Human Th2 Reagent 1 | 967539 | 1 vial | May be stored at 2-8 °C under sterile conditions for up to 3 months.*
Human Th2 Reagent 2 | 967560 | 1 vial | May be stored at 2-8 °C under sterile conditions for up to 3 months.*
Human Th2 Reagent 3 | 967561 | 1 vial | May be stored at 2-8 °C under sterile conditions for up to 3 months.*
Human Th2 Reagent 4 | 967562 | 1 vial | May be stored at 2-8 °C under sterile conditions for up to 3 months.*
Reconstitution Buffer 1 | 967535 | 2 vials | * Provided this is within the expiration date of the kit.
Reconstitution Buffer 2 | 967533 | 2 vials |
20X Wash Buffer | 967557 | 3 vials |

* Other materials & supplies required:
- Ficoll-Hypaque™
- MagCellect™ Human Naive CD4+ T Cell Isolation Kit (R&D Systems, Catalog # MAGH115, or equivalent).
- X-VIVO™15 Chemically Defined, Serum-free Hematopoietic Cell Medium (Lonza, or equivalent)
- Penicillin/Streptomycin (optional)
- Sterile deionized water
- Monensin (Tocris, Catalog # 5223)
- PMA (Tocris, Catalog # 1201)
- Ionomycin (Tocris, Catalog # 1704)
- Tissue culture flasks and/or plates
- Pipettes and pipette tips
- Inverted microscope
- Hemocytometer
- 37 °C, 5% CO₂ incubator
- Centrifuge

**REAGENT PREPARATION**

**Human Th2 Differentiation Media**

1. Reconstitute Human Th2 Reagent 1 and Human Th2 Reagent 2 each with 150 µL of Reconstitution Buffer 1, this is a 1000X stock.
2. Reconstitute Human Th2 Reagent 3 and Human Th2 Reagent 4 each with 150 µL of Reconstitution Buffer 2, this is a 1000X stock.
3. Add 25 µL each of Human Th2 Reagents 1, 2, 3, and 4 to 24.9 mL of cell culture media (X-VIVO 15 medium, 100 units/mL Penicillin, and 100 µg/mL Streptomycin).

**Human CD3 Antibody**

1. Reconstitute the Mouse Anti-Human CD3 antibody with 150 µL of Reconstitution Buffer 2, this is a 100X stock.
2. Add 10 mL of 20X Wash Buffer to 190 mL of sterile deionized water to prepare 200 mL of 1X Wash Buffer.
3. Just before coating, dilute the 100X antibody stock 100-fold with 1X Wash Buffer.

**PROTOCOL FOR TH2 DIFFERENTIATION**

1. Coat a plate with Mouse Anti-Human CD3 antibody.
   a. For a 24-well plate, add 250 µL/well of diluted CD3 antibody.
   b. Incubate at 2-8 °C overnight.
   c. Wash the plate with 1X Wash Buffer twice before use.
2. Isolate human peripheral blood mononuclear cells (PBMCs) from human blood using Ficoll-Hypaque density gradient centrifugation.
3. Isolate human naïve CD4 T cells from human PBMCs using the MagCellect Human Naive CD4 T Cell Isolation Kit.
4. Suspend human naïve CD4 T cells at 1-2 x 10⁵ cells/mL in Human Th2 Differentiation Media.
5. Add the cells to a human CD3 antibody-coated plate. For a 24-well plate, add 1 mL/well. For a 96-well plate, add 0.2 mL/well.
6. Incubate the cells in a 37 °C, 5% CO₂ humidified incubator for 1 hour. Then add Ionomycin (Tocris, Catalog # 1704), PMA (Tocris, Catalog # 1201), and monensin (Tocris, Catalog # 5223). Incubate the cells in a humidified incubator for 6 hours. Analyze cytokine expression via flow cytometry.
7. After 13 days of differentiation, the differentiated Th2 cells are ready to be used in the desired application.
9. To verify Th2 cell differentiation via ELISA, remove the supernatant on day 13 and analyze via ELISA.
10. To verify Th2 cell differentiation via flow cytometry, wash the cells with X-VIVO15 medium once, reuspend the cells in 1 mL X-VIVO 15 medium, 100 units/mL penicillin, 100 µg/mL streptomycin, 50 ng/mL PMA, and 1 µg/mL Ionomycin. Incubate the cells in a 37 °C, 5% CO₂ humidified incubator for 1 hour. Then add monensin at 3 µM and incubate for 6 hours. Analyze cytokine expression via flow cytometry.
11. To verify Th2 cell differentiation via ELISA, remove the supernatant on day 13 and analyze via ELISA.

**PROTOCOL OUTLINE**

1. Coat wells of a 24-well plate with Mouse Anti-Human CD3 Antibody.
2. Isolate PBMCs from human blood.
3. Perform a cell count.
4. Suspend 1-2 x 10⁵ naïve CD4 T cells/mL in Human Th2 Differentiation Media. Culture the cells on plates pre-coated with CD3 antibody for 13 days.
5. Refresh the Differentiation Media every 3-4 days.
6. Re-stimulate the cells with mitogens.
7. Verify Th2 cell differentiation by analyzing cytokine expression via flow cytometry.

---

*All trademarks and registered trademarks are the property of their respective owners.*