DATA EXAMPLES

Differentiation of naïve 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.

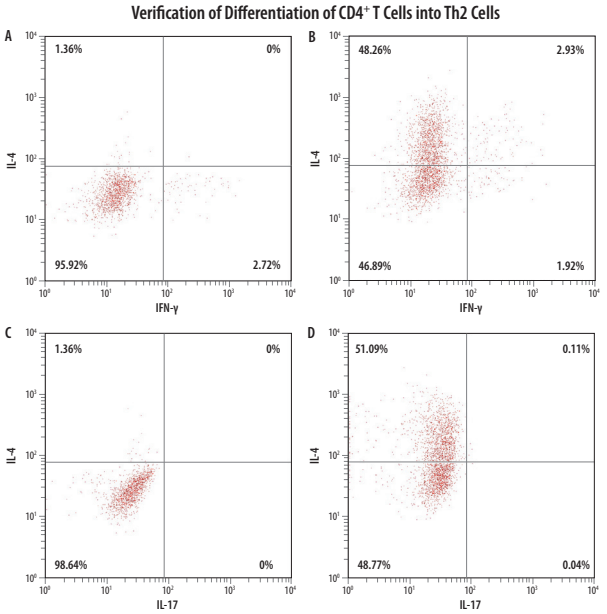

Figure 1: Intracellular Cytokine Staining of Differentiated Human Th2 Cells. Flow cytometry data showing human peripheral blood naïve CD4$^+$ T cells without (A, C) and with (B, D) a 13 day differentiation using reagents included in the Human Th2 Cell Differentiation Kit. On day 13 of differentiation, 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

CATALOG #	DESCRIPTION
IC285A, and IC0041A	Human IFN-γ APC MAb (Clone 25723), Mouse IgG_{2B}, and Mouse IgG_{2B} APC Isotype Control (Clone 133303)
IC204P, and IC002P	Human IL-4 Phycoerythrin MAb (Clone 3007), Mouse IgG_1 and Mouse IgG_1 Phycoerythrin Isotype Control (Clone 11711)
IC3171C, and IC002C	Human IL-17 PerCP MAb (Clone 41802), Mouse IgG_1, and Mouse IgG_1 PerCP Isotype Control (Clone 11711)
FAB3791F, and IC003F	Human CD4 Fluorescein MAb (Clone 11830), Mouse IgG_{2A}, and Mouse IgG_{2A} Fluorescein Isotype Control (Clone 20102)
FC004	Flow Cytometry Fixation Buffer (1X)
FC005	Flow Cytometry Permeabilization/Wash Buffer (1X)

DATA EXAMPLES *CONTINUED*

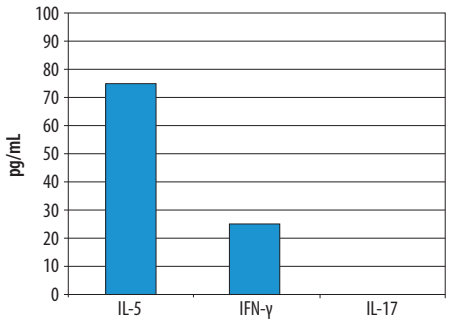

Figure 2: Differentiated Human CD4$^+$ T Cells Secrete IFN-γ. Human peripheral blood naïve CD4$^+$ T cells were differentiated for 13 days under Th2 polarization conditions using reagents included in the Human Th2 Cell Differentiation Kit. On day 13, cell culture supernatant was collected and cytokine secretion was determined using the Human IL-5 Quantikine ELISA Kit, the Human IFN-γ Quantikine ELISA Kit, and the Human IL-17 Quantikine ELISA Kit. All relevant R&D Systems Quantikine ELISA Kits and corresponding catalog numbers are listed below.

SUGGESTED REAGENTS FOR ELISA

CATALOG #	DESCRIPTION
D5000B, or DY205	Human IL-5 Quantikine ELISA Kit, or Human IL-5 DuoSet®
DIF50, or DY285	Human IFN-γ Quantikine ELISA Kit, or Human IFN-γ DuoSet
D1700, or DY317	Human IL-17 Quantikine ELISA Kit, or Human IL-17 DuoSet

REFERENCES

1. Li, Z. *et al.* (2013) Prot. Cell **2**:604.
2. Luckheeram, R.V. *et al.* (2012) Clin. Dev. Immunol. **2012**:925135.
3. Hirahara, K. *et al.* (2011) Immunology **134**:235.

CellXVivo™

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 IL17E/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.

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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	967558	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	967559	1 vial	
Human Th2 Reagent 2	967560	1 vial	
Human Th2 Reagent 3	967561	1 vial	
Human Th2 Reagent 4	967562	1 vial	
Reconstitution Buffer 1	967552	2 vials	May be stored under sterile conditions for up to 3 months at 2-8 °C.*
Reconstitution Buffer 2	967553	2 vials	
20X Wash Buffer	967557	3 vials	

* Provided this is within the expiration date of the kit.

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% CO_2 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. For a 96-well plate, add 50 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 Naïve CD4+ T Cell Isolation Kit.
4. Suspend human naïve CD4+ T cells at 1-2 x 10^5 cells/mL in Human Th2 Differentation 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% CO_2 humidified incubator for 13 days. Refresh the Human Th2 Differentiation Media every 3-4 days according to step 7.
7. Refresh the Human Th2 Differentiation Media by removing 900 µL of the media from each well of a 24-well plate or 180 µL of the media from each well of a 96-well plate and replenishing with the same volume of fresh Human Th2 Differentiation Media every 3-4 days.

   Note: *When refreshing the media, if the cell culture media turns yellow or the cell density reaches 1.5 x 10^6 cells/mL, the cells need to be split. The first split should be at a 1:10 dilution and subsequent splits at 1:2.*
8. 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, resuspend 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% CO_2 humidified incubator for 1 hour. Then add monensin at 3 µM and incubate for 6 hours. Analyze cytokine expression via flow cytometry.

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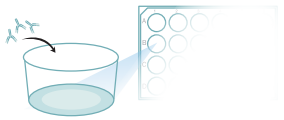
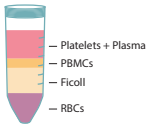
PROTOCOL OUTLINE

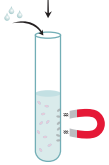
Coat wells of a 24-well plate with Mouse Anti-Human CD3 Antibody.



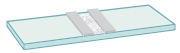
Isolate PBMCs from human blood.



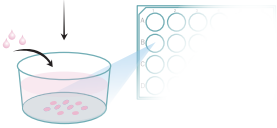
Isolate human naïve CD4+ T cells from PBMCs (e.g., using magnetic cell selection).



Perform a cell count.

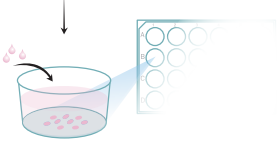


Suspend 1-2 x 10^5 naïve CD4+ T cells/mL in Human Th2 Differentiation Media. **Culture** the cells on plates pre-coated with CD3 antibody for 13 days.



Refesh the Differentiation Media every 3-4 days.

Re-stimulate the cells with mitogens.



Verify Th2 cell differentiation by analyzing cytokine expression via flow cytometry.