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.

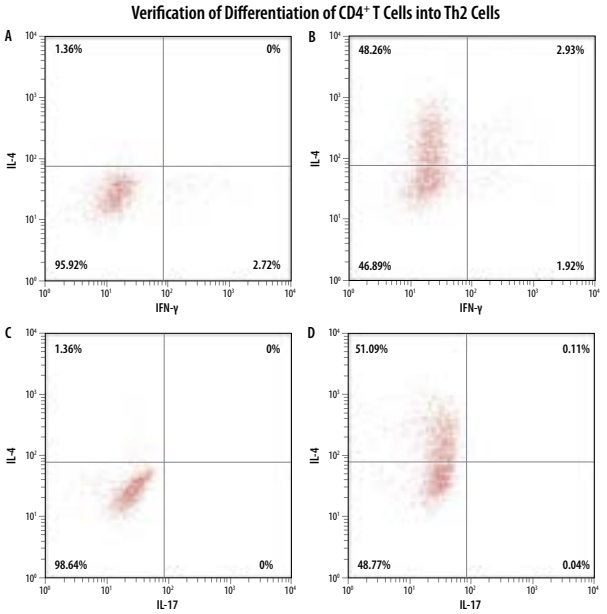

Figure 1: Intracellular Cytokine Staining of Differentiated Human Th2 Cells. Flow cytometry data showing human peripheral blood naïve CD4$^+$ T cells without (A, C) and with (B, D) a 13 day differentiation using reagents included in the Human Th2 Cell Differentiation Kit. On day 13 of differentiation,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

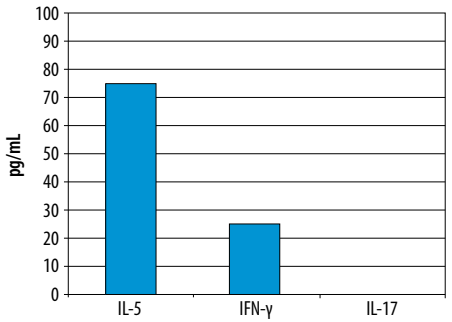

Figure 2: Differentiated Human CD4$^+$ T Cells Secrete IL-5. Human peripheral blood naïve CD4$^+$ T cells were differentiated for 13 days under Th2 polarization conditions using reagents included in the Human Th2 Cell Differentiation Kit. On day 13, cell culture supernatant was collected and cytokine 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® 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® ELISA
DIF50, or DY285	Human IFN-γ Quantikine® ELISA Kit, or Human IFN-γ DuoSet® ELISA
D1700, or DY317	Human IL-17 Quantikine® ELISA Kit, or Human IL-17 DuoSet® ELISA

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 the necessary components to differentiate approximately 5x10^6 naïve CD4$^+$ T cells, and generate 2.5x10^7 CD4$^+$ cells of which 30-50% are IL-4$^+$ 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
- Cell Activation Cocktail 500X (Tocris®, Catalog #5476)
- Tissue culture flasks and/or plates
- Pipettes and pipette tips
- Inverted microscope
- Hemocytometer
- 37 °C, 5% CO_2 incubator
- Centrifuge

REAGENT PREPARATION

Human Th2 Reagent 1 (1000X) – Add 150 µL of Reconstitution Buffer 1 to produce Human Th2 Reagent 1 (1000X).

Human Th2 Reagent 2 (1000X) – Add 150 µL of Reconstitution Buffer 1 to produce Human Th2 Reagent 2 (1000X).

Human Th2 Reagent 3 (1000X) – Add 150 µL of Reconstitution Buffer 2 to produce Human Th2 Reagent 3 (1000X).

Human Th2 Reagent 4 (1000X) – Add 150 µL of Reconstitution Buffer 2 to produce Human Th2 Reagent 4 (1000X).

Human Th2 Differentiation Media – Add Human Th2 Reagent 1 (1000X), Human Th2 Reagent 2 (1000X), Human Th2 Reagent 3 (1000X), Human Th2 Reagent 4 (1000X) to a final concentration of 1X in the desired amount of X-VIVO™ 15 Medium. Addition of Penicillin (100 units/mL) and Streptomycin (100 µg/mL) is recommended. Make fresh as needed.

Mouse Anti-Human CD3 (100X) – Add 150 µL of Reconstitution Buffer 2 to produce Mouse Anti-Human CD3 (100X).

Wash Buffer (1X) – Dilute 20X Wash Buffer 1:20 in sterile deionized water to produce Wash Buffer (1X).

Mouse Anti-Human CD3 (1X) – Dilute Mouse Anti-Human CD3 (100X) 1:100 with Wash Buffer (1X) to produce Mouse Anti-Human CD3 (1X).

PROTOCOL FOR Th2 DIFFERENTIATION

1. Coat the desired tissue culture plate with Mouse Anti-Human CD3 (1X).
   a. Add Mouse Anti-Human CD3 (1X) to plate using the suggested coating volumes below.
   b. Incubate at 2-8 °C overnight.
   c. Wash the plate with Wash Buffer (1X) twice before use.

PLATE	SUGGESTED COATING VOLUME
24-well plate	0.25 mL/well
96-well plate	0.2 mL/well

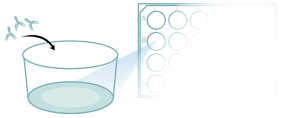
2. Isolate human peripheral blood mononuclear cells (PBMCs) from human blood using Ficoll-Hypaque density gradient centrifugation.
3. Isolate human naïve CD4+ T cells from human PBMCs using the MagCellect™ Human Naïve CD4+ T Cell Isolation Kit.
4. Suspend human naïve CD4+ T cells at 1-2 x 10^5 cells/mL in Human Th2 Differentiation Media.
5. Add the cells to a Mouse Anti-Human CD3 Antibody-Coated Plate.

PLATE	SUGGESTED VOLUME
24-well plate	1.0 mL/well
96-well plate	0.2 mL/well

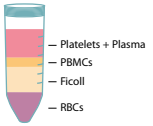
6. Incubate the cells in a 37 °C, 5% CO_2 humidified incubator for 13 days. Refresh the Human Th2 Differentiation Media every 3-4 days according to step 7.
7. Refresh the Human Th2 Differentiation Media by removing 900 µL of the media from each well of a 24-well plate or 180 µL of the media from each 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. On day 13 of differentiation, the differentiated Th2 cells are ready to be used for downstream applications.
9. To verify Th2 cell differentiation via ELISA, remove and analyze the supernatant on day 13 of culture.
10. To verify Th2 cell differentiation via flow cytometry, wash the cells with X-VIVO™15 medium once, resuspend the cells in 1.0 mL X-VIVO™15 medium, 100 units/mL penicillin, 100 µg/mL streptomycin, and 1X Cell Activation Cocktail. Incubate the cells in a 37 °C, 5% CO_2 humidified incubator for 7-8 hours. Analyze cytokine expression via flow cytometry.

PROTOCOL OUTLINE

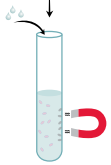
Coat wells of a 24-well plate with Mouse Anti-Human CD3 Antibody.



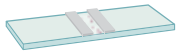
Isolate PBMCs from human blood.



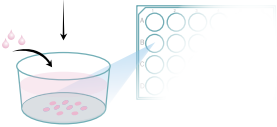
Isolate human naïve CD4+ T cells from PBMCs (e.g., using magnetic cell selection).



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 Mouse Anti-Human CD3 antibody for 13 days.



Refesh the Human Th2 Differentiation Media every 3-4 days.

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