DATA EXAMPLES

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

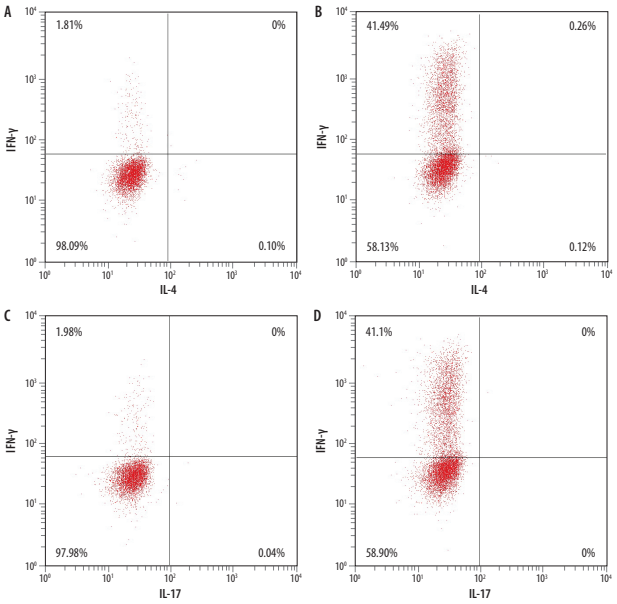

Figure 1: Intracellular Cytokine Staining of Differentiated Human Th1 cells. Flow cytometry data showing human peripheral blood naïve CD4$^+$ T cells without (A, C) and with (B, D) differentiation using reagents included in the Human Th1 Cell Differentiation Kit. (A, B) The cells were stained with a Human IFN-γ APC Monoclonal Antibody and a Human IL-4 PE Monoclonal Antibody. (C, D) The cells were stained with a Human IFN-γ APC Monoclonal Antibody and a Human IL-17 PerCP Monoclonal Antibody. Control cultures were used to place the quandrants. 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*

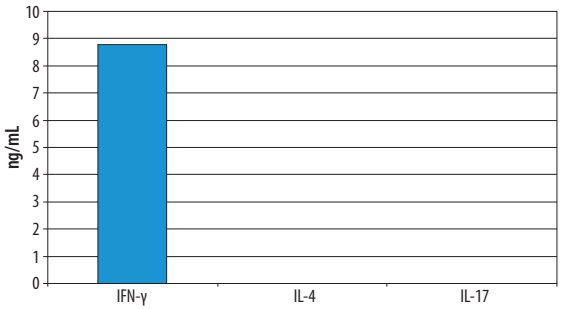

Figure 2: Differentiated Human CD4$^+$ T Cells Secrete IFN-γ. Human peripheral blood naïve CD4$^+$ T cells were cultured using reagents included in the Human Th1 Cell Differentiation Kit. Cytokine expression was determined using the Human IFN-γ Quantikine™ ELISA Kit, the Human IL-4 Quantikine ELISA Kit, 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
DIF50, or DY285	Human IFN-γ Quantikine ELISA Kit, or Human IFN-γ DuoSet™ ELISA
D4050, or DY204	Human IL-4 Quantikine ELISA Kit, or Human IL-4 DuoSet ELISA
D1700, or DY317	Human IL-17 Quantikine ELISA Kit, or Human IL-17 DuoSet ELISA

REFERENCES

1. Zhu, J. and W.E. Paul (2010) Immunol. Rev. **238**:247.
2. Dardalhon, V. *et al.* (2008) J. Autoimmun. **31**:252.
3. Seder, R.A. and W.E. Paul (1994) Annu. Rev. Immonol. **12**:635.

CellXVivo™

Human Th1 Cell Differentiation Kit

Catalog Number: CDK001

BACKGROUND

T helper type 1 (Th1) cells are a lineage of CD4$^+$ effector T cells that promote cell-mediated immune responses against intracellular viral and bacterial pathogens (1). Enhanced Th1 responses are associated with autoimmune diseases including rheumatoid arthritis, multiple sclerosis, inflammatory bowel disease, and type-1 diabetes (2). Differentiation of CD4$^+$ effector T cells into the Th1 lineage is promoted by cytokines such as IL-12 and IFN-γ (3). Th1 cells secrete IFN-γ, IL-10, and TNF-α. The CellXVivo Human Th1 Differentiation Kit contains the necessary components to differentiate approximately 5 x 10^6 naïve CD4$^+$ T cells, and generate 2.5 x 10^7 CD4$^+$ cells of which 50-70% are IFN-γ$^+$ Th1 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	967554	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 Th1 Reagent 1	967555	1 vial	
Human Th1 Reagent 2	967556	1 vial	
Reconstitution Buffer 1	967552	1 vial	May be stored under sterile conditions for up to 3 months at 2-8 °C.*
Reconstitution Buffer 2	967553	1 vial	
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).
- RPMI 1640
- Fetal Bovine Serum (FBS)
- L-Glutamine/Penicillin/Streptomycin (optional)
- β-Mercaptoethanol (2-ME)
- 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 Th1 Reagent 1 (200X) – Add 250 µL of Reconstitution Buffer 1 to produce Human Th1 Reagent 1 (200X).

Human Th1 Reagent 2 (200X) – Add 250 µL of Reconstitution Buffer 1 to produce Human Th1 Reagent 2 (200X).

Human Th1 Differentiation Media – Add Human Th1 Reagent 1 (200X) and Human Th1 Reagent 2 (200X) to a final concentration of 1X in the desired amount of cell culture media (RPMI 1640 with 5% FBS, 2 mM L-Glutamine, and 50 µM 2-ME). Addition of Penicillin (50 units/mL) and Streptomycin (50 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 Th1 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	0.05 mL/well

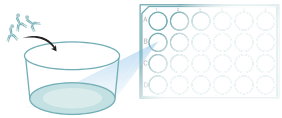
2. Isolate human peripheral blood mononuclear cells (PBMCs) from human blood using Ficoll-Hypaque density gradient centrifugation.
3. Isolate human naïve CD4$^+$ T cells from human PBMCs using the MagCellect Human Naïve CD4$^+$ T Cell Isolation Kit or equivalent.
4. Suspend human naïve CD4$^+$ T cells at 2-4 x 10^5 cells/mL in Human Th1 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

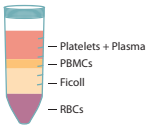
6. Incubate the cells in a 37 °C, 5% CO_2 humidified incubator for up to 10 days. Top off cultures with the same volume of Th1 Differentiation Media used for seeding.
7. Inspect the cultures daily for media exhaustion. When the media changes color between 8-10 days of cultivation, the differentiated Th1 cells are ready to be used for downstream applications.
8. To verify Th1 cell differentiation via ELISA, remove and analyze the supernatant.
9. To verify Th1 cell differentiation via flow cytometry, wash the cells with RPMI 1640 once, then resuspend the cells in 1.0 mL of RPMI 1640 containing 10% FBS, 2 mM L-Glutamine, and 1X Cell Activation Cocktail. Addition of Penicillin (50 units/mL) and Streptomycin (50 µg/mL) is recommended. Incubate the cells in a 37 °C, 5% CO_2 humidified incubator for 4 hours. Analyze cytokine expression by flow cytometry.

PROTOCOL OUTLINE

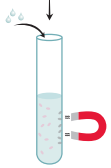
Coat wells of a plate with Mouse Anti-Human CD3 Antibody.



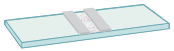
Isolate PBMCs from human blood.



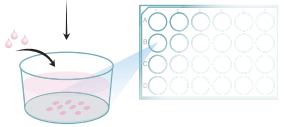
Isolate human naïve CD4$^+$ T cells from PBMCs (e.g., using magnetic cell selection).



Perform a cell count.



Suspend 2-4 x 10^5 naïve CD4$^+$ T cells/mL in Human Th1 Differentiation Media.
Culture the cells on plates pre-coated with Mouse Anti-Human CD3 antibody.



Verify Th1 cell differentiation by analyzing cytokine expression via flow cytometry.