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.

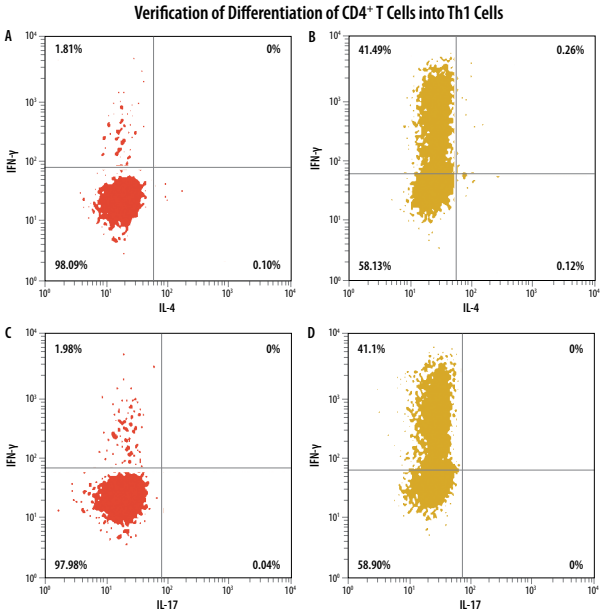

Figure 1: Differentiated Human CD4$^+$ T Cells Stain for IFN-γ.
Flow cytometry data showing human peripheral blood naïve CD4$^+$ T cells without (A, C) and with (B, D) a 5 day differentiation using reagents included in the Human Th1 Cell Differentiation Kit. (A, B) The cells were stained with a Human 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*

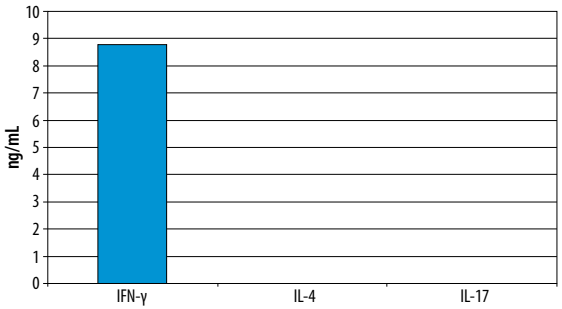

Figure 2: Differentiated Human CD4$^+$ T Cells Secrete IFN-γ.
Human peripheral blood naïve CD4$^+$ T cells were cultured for 5 days using reagents included in the Human Th1 Cell Differentiation Kit. Cytokine expression was determined using the Human IFN-γ Quantikine ELISA Kit, the Human IL-4 Quantikine ELISA kit, and the Human IL-17 Quantikine ELISA Kit. All relevant R&D Systems Quantikine 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®
D4050, or DY204	Human IL-4 Quantikine ELISA Kit, or Human IL-4 DuoSet
D1700, or DY317	Human IL-17 Quantikine ELISA Kit, or Human IL-17 DuoSet

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 Human Th1 Differentiation Kit contains all necessary components to differentiate human naïve CD4$^+$ T cells into 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

- MagCellect™ Human Naïve CD4+ T Cell Isolation Kit (R&D Systems, Catalog # MAGH115, or equivalent).
- Monesin
- PMA
- Ficoll-Hypaque™
- RPMI 1640
- L-Glutamine
- Ionomycin
- Penicillin
- Streptomycin
- Fetal Bovine Serum (FBS)
- β-Mercaptoethanol (2-ME)
- Tissue culture flasks and/or plates
- Microscope
- Hemocytometer
- 37 °C, 5% CO_2 incubator
- Centrifuge
- Pipettes and pipette tips

REAGENT PREPARATION

Human Th1 Differentiation Media

1. Reconstitute Human Th1 Reagent 1 with 250 µL of Reconstitution Buffer 1, this is a 200X stock.
2. Reconstitute Human Th1 Reagent 2 with 250 µL of Reconstitution Buffer 1, this is a 200X stock.
3. Add 50 µL of Human Th1 Reagent 1 and 50 µL of Human Th1 Reagent 2 to 9.9 mL of cell culture media (RPMI, 2 mM L-Glutamine, 50 units/mL Penicillin, 50 µg/mL Streptomycin, 5% FBS, and 50 µM 2-ME).

Human CD3 Antibody

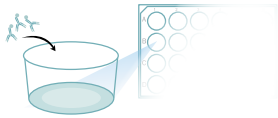
1. Reconstitute the Mouse Anti-Human CD3 antibody with 150 µL of Reconstitution Buffer 2, this is a 100X stock.
2. Add a 10 mL of 20X Wash Buffer to 190 mL of sterile deionized water to prepare 200 mL of 1X Wash Buffer.
3. Just before coating, dilute the 100X antibody stock 100-fold with 1X Wash Buffer.

PROTOCOL FOR Th1 DIFFERENTIATION

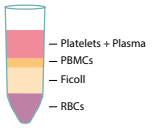
1. Coat a plate with Mouse Anti-Human CD3 antibody.
   a. For a 24-well plate, add 250 µL/well of diluted CD3 antibody. For a 96-well plate, add 50 µL/well of diluted CD3 antibody.
   b. Incubate at 2-8 °C overnight.
   c. Wash the plate with 1X Wash Buffer twice before use.
2. Isolate human peripheral 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 Th1 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 5 days.
7. Collect media to analyze cytokine production profile.
8. Wash the cells once with RPMI, resuspend the cells in 1 mL of RPMI, 2 mM L-Glutamine, 50 units/mL penicillin, 50 µg/mL streptomycin, 10% FBS, 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 monesin at 3 µM and incubate for 3 hours.
9. Analyze cytokine expression via flow cytometry.

PROTOCOL OUTLINE

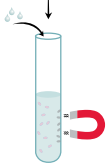
Coat wells of a plate with Mouse Anti-Human CD3 Antibody.



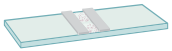
Isolate PBMCs from human blood.



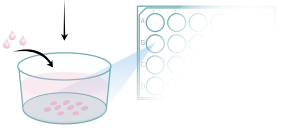
Isolate human naïve CD4+ T cells from PBMCs (e.g., using magnetic cell selection).



Perform a cell count.



Suspend 1-2 x 10^5 naïve CD4+ T cells/mL in differentiation media supplemented with Human Th1 Reagents 1 and 2. **Culture** the cells on plates pre-coated with CD3 antibody.



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