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.

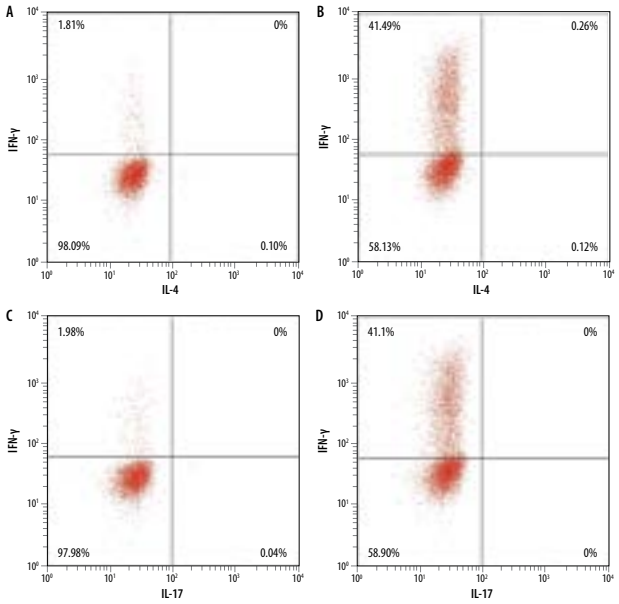


Figure 1: Intracellular Cytokine Staining of Differentiated Human Th1 cells. Flow cytometry data showing human peripheral blood naïve CD4$^+$ T cells without (A, C) and with (B, D) a 5 day differentiation using reagents included in the Human Th1 Cell Differentiation Kit. (A, B) The cells were stained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

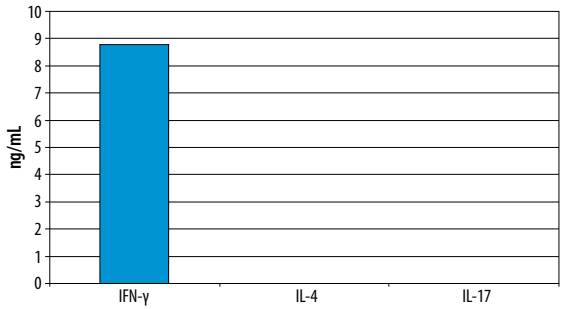


Figure 2: Differentiated Human CD4$^+$ T Cells Secrete IFN-γ. Human peripheral blood naïve CD4$^+$ T cells were cultured for 5 days using reagents included in the Human Th1 Cell Differentiation Kit. Cytokine expression was determined using the Human IFN-γ Quantikine® ELISA Kit, the Human IL-4 Quantikine®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

Manufactured and Distributed by:

USA R&D Systems, Inc.
614 McKinley Place NE, Minneapolis, MN 55413
TEL: 800 343 7475 612 379 2956 FAX: 612 656 4400
E-MAIL: info@bio-techne.com

Distributed by:

Europe | Middle East | Africa Bio-Techne Ltd.
19 Barton Lane Abingdon Science Park, Abingdon OX14 3NB, UK
TEL: +44 (0)1235 529449 **FAX:** +44 (0)1235 533420
E-MAIL: info.emea@bio-techne.com

China Bio-Techne China Co., Ltd.
Unit 1901, Tower 3, Raffles City Changning Office,
1193 Changning Road, Shanghai PRC 200051
TEL: +86 (21) 52380373 (400) 821-3475 **FAX:** +86 (21) 52371001
E-MAIL: info.cn@bio-techne.com

www.RnDSystems.com

R&D SYSTEMS
a bio-techne brand

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

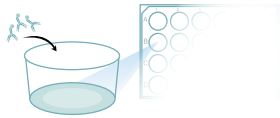
2. Isolate human peripheral blood mononuclear cells (PBMCs) from human blood using Ficoll-Hypaque density gradient centrifugation.
3. Isolate human naïve CD4+ T cells from human PBMCs using the MagCellect™ Human Naïve CD4+ T Cell Isolation Kit.
4. Suspend human naïve CD4+ T cells at 1-2 x 10^5 cells/mL 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

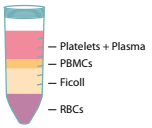
6. Incubate the cells in a 37 °C, 5% CO_2 humidified incubator for 5 days.
7. On day 5 of differentiation, the differentiated Th1 cells are ready to be used for downstream applications.
8. To verify Th1 cell differentiation via ELISA, remove and analyze the supernatant on day 5 of culture.
9. To verify 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

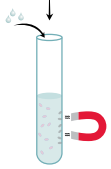
Coat wells of a plate with Mouse Anti-Human CD3 Antibody.



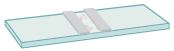
Isolate PBMCs from human blood.



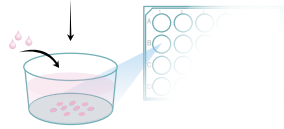
Isolate human naïve CD4+ T cells from PBMCs (e.g., using magnetic cell selection).



Perform a cell count.



Suspend 1-2 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.