DATA EXAMPLES

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

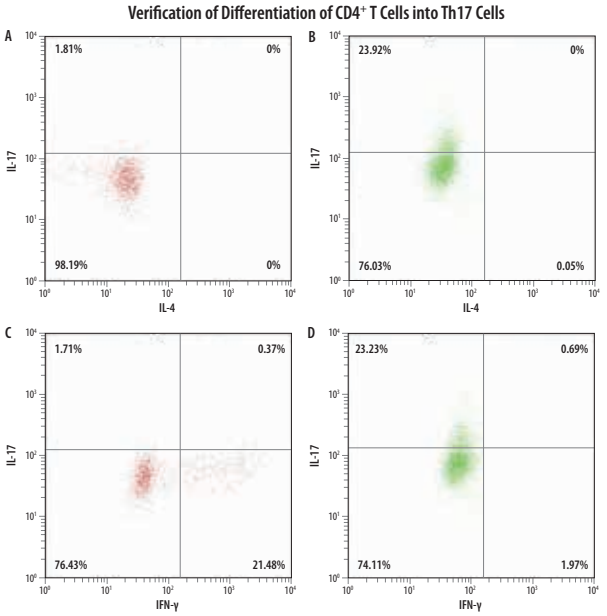


Figure 1: Intracellular Cytokine Staining of Differentiated Human Th17 Cells. Flow cytometry data showing human peripheral blood naïve CD4$^+$ T cells without (A, C) and with (B, D) a 10 day differentiation using reagents included in the Human Th17 Cell Differentiation Kit. On day 10 of differentiation, 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
IC317A , and IC0041A	Human IL-17 APC MAb (Clone 41809), Mouse IgG_{2B}, and Mouse IgG_{2B} APC Isotype Control (Clone 133303)
IC285P, and IC0041P	Human IFN-γ PE (Clone 25723), Mouse IgG_{2B}, and Mouse IgG_{2B} PE Isotype Control (Clone 133303)
IC204F, and IC002F	Human IL-4 Fluorescein MAb (Clone 3007), Mouse IgG_1, and Mouse IgG_1 Fluorescein Isotype Control (Clone 11711)
FAB3791C, and IC003C	Human CD4 PerCP MAb (Clone 11830), Mouse IgG_{2A}, and Mouse IgG_{2A} PerCP Isotype Control (Clone 20102)
FC004	Flow Cytometry Fixation Buffer (1X)
FC005	Flow Cytometry Permeabilization/Wash Buffer (1X)

DATA EXAMPLES *CONTINUED*

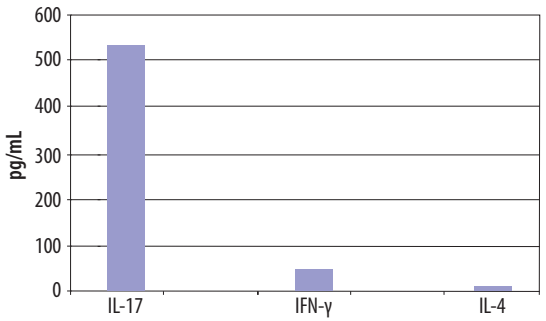


Figure 2: Differentiated Human CD4$^+$ Cells Secrete IL-17. Human peripheral blood naïve CD4$^+$ T cells were differentiated for 10 days under Th17 polarization conditions using reagents included in the Human Th17 Cell Differentiation Kit. On day 10, cell culture supernatant was collected and cytokine secretion was determined using the Human IL-17 Quantikine ELISA Kit, the Human IFN-γ Quantikine ELISA Kit, and the Human IL-4 Quantikine ELISA Kit. All relevant R&D Systems Quantikine ELISA kits and corresponding catalog numbers are listed below.

SUGGESTED REAGENTS FOR ELISA

CATALOG #	DESCRIPTION
D1700, or DY317	Human IL-17 Quantikine ELISA Kit, or Human IL-17 DuoSet®
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

REFERENCES

1. Sundrud, M.S. and C. Trivigno (2013) Semin. Immunol. **25**:263.
2. Luckheeram, R.V. *et al.* (2012) Clin. Dev. Immunol. **2012**:925135.
3. Hirahara, K. *et al.* (2011) Immunology **134**:235.

CellXVivo™

Human Th17 Cell Differentiation Kit

Catalog Number: CDK003B

BACKGROUND

T helper type 17 (Th17) cells are a lineage of CD4$^+$ effector T cells that protect against extracellular bacteria and fungi. They function as pro-inflammatory agents by recruiting other inflammatory immune cells and opposing some regulatory T cell (Treg) functions. Th17 cells also mediate autoimmune and inflammatory disease pathogenesis (1). Differentiation of CD4$^+$ effector T cells into the Th17 lineage is promoted by cytokines such as TGF-β and IL-6, while their survival and expansion are dependent on IL-21 and IL-23 (2, 3). Th17 cells secrete TNF-α, IL-6, IL-9, IL-17A, IL-17F, IL-21, IL-22, and (human) IL-26/AK155. The CellXVivo Human Th17 Cell Differentiation Kit contains anti-human CD3 and CD28 antibodies and Th17 differentiation activators, which drive naïve CD4$^+$ T cells into Th17 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 & Canada | R&D Systems, Inc.
614 McKinley Place NE, Minneapolis, MN 55413, USA
TEL: (800) 343-7475 (612) 379-2956 FAX: (612) 656-4400
E-MAIL: info@RnDSystems.com

DISTRIBUTED BY:
UK & Europe | R&D Systems Europe, Ltd.
19 Barton Lane, Abingdon Science Park, Abingdon OX14 3NB, UK
TEL: +44 (0)1235 529449 FAX: +44 (0)1235 533420
E-MAIL: info@RnDSystems.co.uk

China | R&D Systems China Co., Ltd.
24A1 Hua Min Empire Plaza, 726 West Yan An Road, Shanghai PRC 200050
TEL: +86 (21) 52380373 FAX: +86 (21) 52371001
E-MAIL: info@RnDSystemsChina.com.cn

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	967563	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.*
Goat Anti-Human CD28	967564	1 vial	
Human Th17 Reagent 1	967565	1 vial	
Human Th17 Reagent 2	967566	1 vial	
Human Th17 Reagent 3	967567	1 vial	
Human Th17 Reagent 4	967568	1 vial	
Human Th17 Reagent 5	967592	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	
Reconstitution Buffer 3	968014	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).
- 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 Th17 Differentiation Media

1. Reconstitute Human Th17 Reagents 1 and 3 each with 250 µL of Reconstitution Buffer 1, this is a 400X stock.
2. Reconstitute Human Th17 Reagent 2 with 250 µL of Reconstitution Buffer 3, this is a 400X stock.
3. Reconstitute Human Th17 Reagents 4 and 5 each with 250 µL of Reconstitution Buffer 2, this is a 400X stock.
4. Add 62.5 µL each of Human Th17 Reagent 1, 2, 3, 4, and 5 to 24.6 mL of cell culture media (X-VIVO 15 medium, 100 units/mL Penicillin, and 100 µg/mL Streptomycin).

Human CD3 and CD28 Antibodies

1. Reconstitute the Mouse Anti-Human CD3 and CD28 antibodies each with 100 µL of Reconstitution Buffer 2, these are 40X stocks.
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 each 40X antibody stock 1:40 with 1X Wash Buffer.

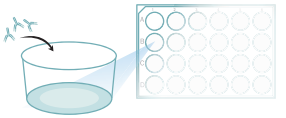
PROTOCOL FOR Th17 DIFFERENTIATION

1. Coat a plate with Mouse Anti-Human CD3 and CD28 antibodies.
   a. For a 24-well plate, add 125 µL/well of diluted CD3 antibody and 125 µL/well of diluted CD28 antibody.
   For a 96-well plate, add 25 µL/well of diluted CD3 antibody and 25 µL/well of diluted CD28 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 Th17 Differentation Media.
5. Add the cells to a human CD3 and CD28 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 10 days. Refresh the Human Th17 Differentiation Media every 2-3 days according to step 7.
7. Refresh the Human Th17 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 Th17 Differentiation Media every 2-3 days.

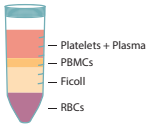
   Note: *When refreshing the media, if the cell culture media turns yellow or the cell density reaches 1.5 x 10^6 cells/mL, the cells need to be split. Split cells at 1:2.*
8. After 10 days of differentiation, the differentiated Th17 cells are ready to be used for downstream applications.
9. To verify Th17 cell differentiation via ELISA, remove the supernatant on day 10 and analyze via ELISA.
10. To verify Th17 cell differentiation via flow cytometry, collect the cells and wash with X-VIVO medium once, resuspend the cells in 1 mL X-VIVO 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 an additional 6 hours. Analyze cytokine expression via flow cytometry.

PROTOCOL OUTLINE

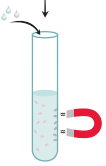
Coat wells of a 24-well plate with Anti-Human CD3 and CD28 Antibodies.



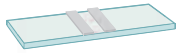
Isolate PBMCs from human blood.



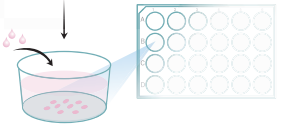
Isolate human naïve $CD4^+$ T cells from PBMCs (e.g., using magnetic cell selection).



Perform a cell count.

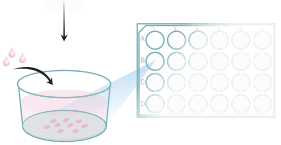


Suspend 1-2 x 10^5 naïve $CD4^+$ T cells/mL in Human Th17 Differentiation Media. **Culture** the cells on plates pre-coated with CD3 and CD28 antibodies for 10 days.



Refesh the Differentiation Media every 2-3 days.

Re-stimulate cells with mitogens.



Verify Th17 cell differentiation by analyzing cytokine expression via flow cytometry.