DATA EXAMPLES

Differentiation of naïve CD4$^+$ T cells into Th17 cells is confirmed by intracellular staining for IL-17 (Figure1) 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.

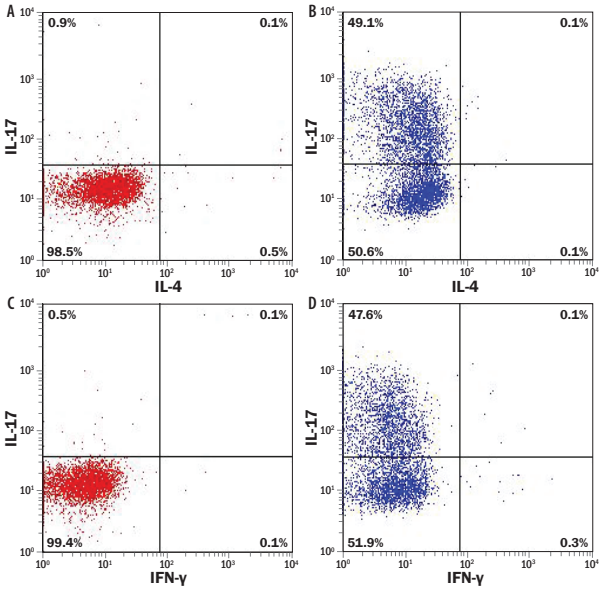

Figure 1: Intracellular Cytokine Staining of Differentiated Mouse Th17 Cells. Flow cytometry data showing mouse naïve CD4$^+$ T cells without (A, C) and with (B, D) a 5 day differentiation using reagents included in this kit. On day 5 of differentiation, the cells were stimulated with Cell Activation Cocktail (Tocris®, Catalog # 5476) and stained with Mouse IL-17, Mouse IFN-γ, and Mouse 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
FAB554A, and IC013A	Mouse CD4 APC-conjugated Antibody (R&D Systems®), and Rat IgG_{2B} APC-conjugated Antibody Isotype Control (R&D Systems®)
NBP1-72027, and IC005P	Mouse IL-17/IL-17A PE-conjugated Antibody (Novus Biologicals®), and Rat IgG_1 PE-conjugated Antibody Isotype Control (R&D Systems®)
IC485A, and IC006A	Mouse IFN-γ APC-conjugated Antibody (R&D Systems®) and Rat IgG_{2A} Allophycocyanin Isotype Control (R&D Systems®)
NBP2-52656, and IC1051A	Mouse IL-4 APC-conjugated Antibody (Novus Biologicals®), and Rabbit IgG APC-conjugated Antibody Isotype Control (R&D Systems®)
FC009	Flow Cytometry Fixation & Permeabilization Buffer Kit 1 (R&D Systems®)
5476	Cell Activation Cocktail 500X (Tocris®)

DATA EXAMPLES *CONTINUED*

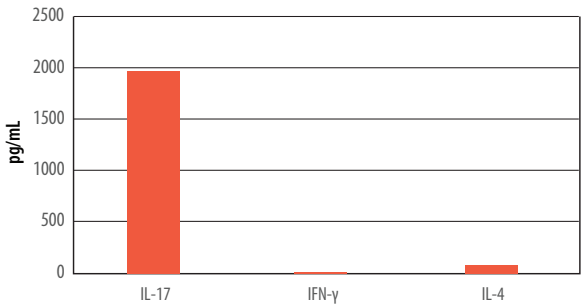

Figure 2: Differentiated Mouse CD4$^+$ T Cells Secrete IL-17. Mouse CD4$^+$ T cells were differentiated for 5 days under Th17 polarization conditions using reagents included in this kit. On day 5, cell culture supernatant was collected and cytokine secretion was determined using the Mouse IL-17 Quantikine® ELISA Kit, the Mouse IFN-γ Quantikine® ELISA Kit, and the Mouse IL-4 Quantikine® ELISA Kit. All relevant R&D Systems® ELISA kits and corresponding catalog numbers are listed below.

SUGGESTED REAGENTS FOR ELISA

CATALOG #	DESCRIPTION
M1700, or DY421	Mouse IL-17 Quantikine® ELISA Kit, or Mouse IL-17 DuoSet® ELISA
MIF00, or DY485	Mouse IFN-γ Quantikine® ELISA Kit, or Mouse IFN-γ DuoSet® ELISA
M4000B, or DY404	Mouse IL-4 Quantikine® ELISA Kit, or Mouse IL-4 DuoSet® ELISA

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™

Mouse Th17 Cell Differentiation Kit

Catalog Number: CDK017

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 IL-26/AK155. The CellXVivo™ Mouse Th17 Cell Differentiation Kit contains anti-mouse CD3 antibody, anti-mouse CD28 antibody, and optimized Th17 differentiation reagents. The quantity of the components in the kit is sufficient to differentiate approximately 50x10^6 naïve CD4$^+$ T cells, and generate ~ 200 x 10^6 cells, of which > 40% are Th17 polarized cells within your starting CD4$^+$ T cell population.

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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R&D SYSTEMS
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MATERIALS PROVIDED & STORAGE CONDITIONS

Store the unopened kit at ≤ -20 °C. Do not use past kit expiration date.

COMPONENTS	PART #	# VIALS	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
Hamster Anti-Mouse CD3	968091	1 vial	May be stored at 2-8 °C under sterile conditions for up to 1 month or at -20 °C to -70 °C in a manual defrost freezer for up to 3 months.*
Rat Anti-Mouse CD28	968096	1 vial	
Mouse Th17 Reagent 1	390604	1 vial	
Mouse Th17 Reagent 2	968092	1 vial	
Mouse Th17 Reagent 3	968093	1 vial	
Mouse Th17 Reagent 4	968094	1 vial	
Mouse Th17 Reagent 5	968095	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	1 vial	
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

- Laboratory mice
- MagCellect™ Mouse Naïve CD4⁺ T Cell Isolation Kit (R&D Systems®, Catalog # MAGM205, 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

Mouse Th17 Reagent 1 (400X) - Add 300 µL of Reconstitution Buffer 1 to Mouse Th17 Reagent 1 to produce Mouse Th17 Reagent 1 (400X).

Mouse Th17 Reagent 2 (400X) - Add 300 µL of Reconstitution Buffer 1 to Mouse Th17 Reagent 2 to produce Mouse Th17 Reagent 2 (400X).

Mouse Th17 Reagent 3 (400X) - Add 300 µL of Reconstitution Buffer 1 to Mouse Th17 Reagent 3 to produce Mouse Th17 Reagent 3 (400X).

Mouse Th17 Reagent 4 (400X) - Add 300 µL of Reconstitution Buffer 1 to Mouse Th17 Reagent 4 to produce Mouse Th17 Reagent 4 (400X).

Mouse Th17 Reagent 5 (400X) - Add 300 µL of Reconstitution Buffer 3 to Mouse Th17 Reagent 5 to produce Mouse Th17 Reagent 5 (400X).

Rat Anti-Mouse CD28 (400X) - Add 300 µL of Reconstitution Buffer 2 to Rat Anti-Mouse CD28 to produce Rat Anti-Mouse CD28 (400X).

Mouse Th17 Differentiation Media - Add Mouse Th17 Reagent 1 (400X), Mouse Th17 Reagent 2 (400X), Mouse Th17 Reagent 3 (400X), Mouse Th17 Reagent 4 (400X), Mouse Th17 Reagent 5 (400X), and Rat Anti-Mouse CD28 (400X) to desired amount of X-VIVO™ 15 Medium. Addition of Penicillin (100 units/mL) and Streptomycin (100 µg/mL) is recommended.

REAGENT PREPARATION *CONTINUED*

Hamster Anti-Mouse CD3 (100X) - Add 400 µL of Reconstitution Buffer 2 to Hamster Anti-Mouse CD3 Antibody to produce Hamster Anti-Mouse CD3 (100X) stock solution.

Wash Buffer (1X) - Add 10 mL of 20X Wash Buffer to 190 mL of sterile deionized water to prepare 200 mL of Wash Buffer (1X).

Hamster Anti-Mouse CD3 (1X) - Dilute Hamster Anti-Mouse CD3 (100X) stock 1:100 with Wash Buffer (1X) to produce Hamster Anti-Mouse CD3 (1X)

PROTOCOL FOR Th17 DIFFERENTIATION

Note: *Results may vary due to strain, age, and/or the health of the mice used for isolation.*

1. Coat the desired tissue culture plate or flask with Hamster Anti-Mouse CD3 (1X).
   a. Add Hamster Anti-Mouse CD3 (1X) to plate or flask using the suggested coating volumes below.
   b. Incubate overnight at 2-8 °C or 2-3 hours at 37 °C.
   c. Wash plate or flask twice with Wash Buffer (1X) just prior to adding cells.

PLATE OR FLASK	SUGGESTED COATING VOLUME	# OF WELLS PER KIT
T-75 cm^2 flask	12 mL/flask	3
T-25 cm^2 flask	4 mL/flask	10
6-well plate	1.6 mL/well	25
24-well plate	0.4 mL/well	100
96-well plate	0.1 mL/well	400

2. Prepare a single cell suspension of mouse splenocytes and isolate mouse naïve CD4⁺ T cells according to the product insert for the MagCellect™ Mouse Naïve CD4⁺ T Cell Isolation Kit. Perform a cell count.
3. Suspend mouse naïve CD4⁺ T cells at 1 x 10^6 cells/mL in Mouse Th17 Differentiation Media.
4. Add the cells to a Hamster Anti-Mouse CD3 antibody-coated plate or flask using the suggested volumes below.

PLATE OR FLASK	SUGGESTED VOLUME
T-75 cm^2 flask	15 mL/flask
T-25 cm^2 flask	5 mL/flask
6-well plate	2 mL/well
24-well plate	0.5 mL/well
96-well plate	0.125 mL/well

5. Incubate the cells in a 37 °C, 5% CO_2 humidified incubator for 3 days.
6. Refresh the Mouse Th17 Differentiation Media on day 3 by adding an equal volume of fresh Mouse Th17 Differentiation Media. Do not remove existing media from the well or flask (i.e add 15 mL fresh Mouse Th17 Differentiation Media directly to a T-75 cm^2 flask).

PROTOCOL FOR Th17 DIFFERENTIATION *CONTINUED*

7. Incubate the cells in a 37 °C, 5% CO_2 humidified incubator for an additional 2 days.
8. On day 5 of differentiation, the differentiated mouse Th17 cells are ready to be used for downstream applications, such as flow cytometry or ELISA.
9. To verify Th17 cell differentiation via flow cytometry, collect the cells and wash with X-VIVO™ 15 Medium once, resuspend the cells to 1-2 x 10^6/mL in X-VIVO™ 15 Medium and Cell Activation Cocktail (1X). Addition of Penicillin (100 units/mL) and Streptomycin (100 µg/mL) is recommended. Incubate the cells in a 37 °C, 5% CO_2, humidified incubator for 6-7 hours. Analyze cytokine expression via flow cytometry.

PROTOCOL OUTLINE