DATA EXAMPLES

Differentiation of naive CD4$^+$ T cells into Th1 cells is confirmed by flow cytometry (Figure 1) and ELISA (Figure 2). These data demonstrate that Th1-polarized cells express and secrete IFN-γ. The corresponding tests for IL-4 (Th2 cell marker) and IL-17 (Th17 cell marker) are low/negative.

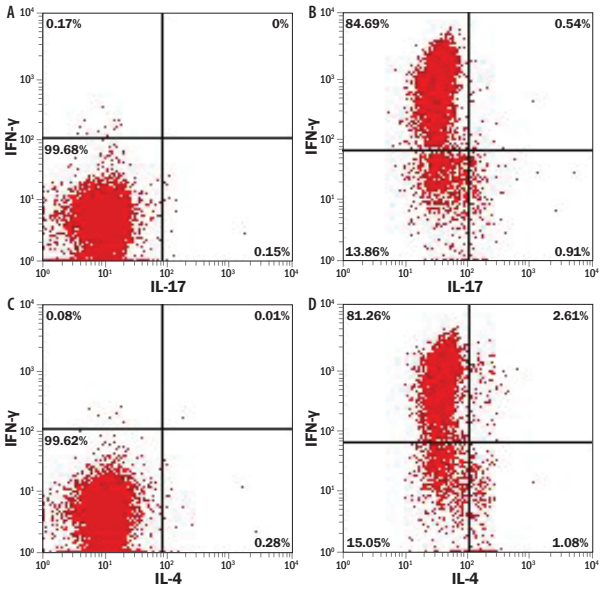

Figure 1: Intracellular Cytokine Staining of Differentiated Mouse Th1 Cells. Flow cytometry data of mouse naïve CD4$^+$ T cells **(A, C)** and differentiated Th1 cells **(B, D)** generated using reagents included in this kit. After 6 days of differentiation, naïve CD4$^+$ cells or differentiated Th1 cells were stimulated with Cell Activation Cocktail (Tocris®, Catalog # 5476) and stained with conjugated Anti-Mouse IFN-γ, Anti-Mouse IL-4 (Clone 11B11), and Anti-Mouse IL-17A Monoclonal Antibodies. Quadrants were set based on isotype-stained controls. All R&D Systems® and Novus Biologicals® antibodies and corresponding catalog numbers used in this figure are shown in the table below.

SUGGESTED REAGENTS FOR FLOW CYTOMETRY

CATALOG #	DESCRIPTION
IC485P , and IC006P	Mouse IFN-γ PE-conjugated Antibody (R&D Systems®), and Rat IgG_{2A} PE-conjugated Antibody (R&D Systems®)
NBP1-72027, and IC006P	Mouse IL-17/IL-17A PE-conjugated Antibody (Novus Biologicals®), and Rat IgG_{2A} PE-conjugated Antibody (R&D Systems®)
FAB554P, and IC005P	Mouse CD4 PE-conjugated Antibody (R&D Systems®), and Rat IgG_1 PE-conjugated Antibody (R&D Systems®)
FC009	Flow Cytometry Fixation & Permeabilization Buffer (R&D Systems®)
5476	Cell Activation Cocktail 500X (Tocris®)

DATA EXAMPLES *CONTINUED*

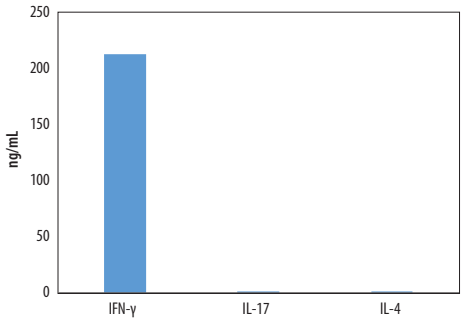

Figure 2: Th1-differentiated Mouse CD4$^+$ Cells Secrete High Levels of IFN-γ. Mouse naïve CD4$^+$ T cells were differentiated for 6 days using the reagents included in this kit. On day 6 of differentiation cells were harvested and re-stimulated with Anti-Mouse CD3 and Anti-Mouse CD28 overnight. The cell culture supernatant was collected and cytokine secretion was determined using the Mouse IFN-γ Quantikine® ELISA Kit, the Mouse IL-4 Quantikine® ELISA Kit, and the Mouse IL-17 Quantikine® ELISA Kit. All relevant Quantikine® and DuoSet® ELISA kits and corresponding R&D Systems® catalog numbers are listed in the table 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. 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. Immunol. **12**:635.

CellXVivo™

Mouse Th1 Cell Differentiation Kit

Catalog Number: CDK018

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™ Mouse Th1 Cell Differentiation Kit contains optimized reagents for Th1 differentiation from naïve CD4$^+$ cells. The quantity of components in this kit is sufficient to differentiate approximately 8 x 10^6 naive CD4$^+$ T cells, and generate 8 x 10^7 CD4$^+$ cells of which ≥ 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 ≤ -20 °C. Do not use past kit expiration date.

COMPONENTS	PART #	# VIALS	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
Hamster Anti-Mouse CD3, Th1	968107	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.*
Mouse Th1 Reagent 1	968108	1 vial	
Mouse Th1 Reagent 2	968109	1 vial	
Mouse Th1 Reagent 3	968110	1 vial	
Mouse Th1 Reagent 4	968111	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	
20X Wash Buffer	967557	1 vial	

* 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 Th1 Reagent 1 (400X) - Add 250 µL of Reconstitution Buffer 1 to Mouse Th1 Reagent 1 to produce Mouse Th1 Reagent 1 (400X).

Mouse Th1 Reagent 2 (400X) - Add 250 µL of Reconstitution Buffer 1 to Mouse Th1 Reagent 2 to produce Mouse Th1 Reagent 2 (400X).

Mouse Th1 Reagent 3 (400X) - Add 250 µL of Reconstitution Buffer 1 to Mouse Th1 Reagent 3 to produce Mouse Th1 Reagent 3 (400X).

Mouse Th1 Reagent 4 (400X) - Add 250 µL of Reconstitution Buffer 2 to Mouse Th1 Reagent 4 to produce Mouse Th1 Reagent 4 (400X).

Mouse Th1 Differentiation Media - Add Mouse Th1 Reagent 1 (400X), Mouse Th1 Reagent 2 (400X), Mouse Th1 Reagent 3 (400X), and Mouse Th1 Reagent 4 (400X) to a final concentration of 1X in the desired amount of X-VIVO™ 15 Medium. Addition of Penicillin (100 units/mL) and Streptomycin (100 µg/mL) is recommended. Make fresh as needed.

Hamster Anti-Mouse CD3 (50X) - Add 100 µL of Reconstitution Buffer 2 to Hamster Anti-Mouse CD3, Th1 Antibody to produce Hamster Anti-Mouse CD3 (50X).

Wash Buffer (1X) - Dilute 20X Wash Buffer 1:20 in sterile deionized water to produce Wash Buffer (1X).

Hamster Anti-Mouse CD3 (1X) - Dilute Hamster Anti-Mouse CD3 (50X) stock 1:50 with Wash Buffer (1X) to produce Hamster Anti-Mouse CD3 (1X).

PROTOCOL FOR Th1 DIFFERENTIATION

The quantity of components in this kit is sufficient to differentiate approximately 8 x 10^6 naïve $CD4^+$ T cells, and generate 8 x 10^7 $CD4^+$ cells of which 70-90% are IFN-γ^+ Th1 polarized cells.

Note: *If starting with fewer cells, adjust starting volumes/number of wells accordingly.*

1. Coat the desired tissue culture plate with Hamster Anti-Mouse CD3 (1X).
   a. Add Hamster Anti-Mouse CD3 (1X) to plate 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	SUGGESTED COATING VOLUME	# OF WELLS PER KIT
24-well plate	0.4 mL/well	8
96-well plate	0.1 mL/well	40

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.
   Note: *1 mouse spleen will provide roughly enough naïve $CD4^+$ T cells for 1 well of a 24-well plate. The quantity of spleens needed may vary based on mouse strain, age, and/or health.*
3. Suspend mouse naïve $CD4^+$ T cells at 1x10^6 cells/mL in Mouse Th1 Differentiation Media.
4. Add the cells to a Hamster Anti-Mouse CD3 antibody-coated plate using the suggested volumes below.

PLATE	SUGGESTED VOLUME
24-well plate	1.0 mL/well
96-well plate	0.2 mL/well

5. Centrifuge the plate at 300 x g for 1 minute and incubate the cells in a 37 °C, 5% CO_2 humidified incubator for 3 days.
6. On day 3 of differentiation, harvest cells and dilute them 1:10 by adding fresh Mouse Th1 Differentiation Media in an appropriate sized conical tube.
7. Transfer diluted cells to a new, uncoated plate or flask using the volumes indicated in the table below. Incubate the cells in a 37 °C, 5% CO_2 humidified incubator for an additional 3 days.

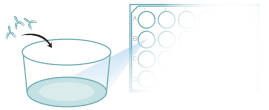
PLATE OR FLASK	SUGGESTED VOLUME
24-well plate	1 mL/well
6-well plate	4 mL/well
T-25	8-10 mL/well
T-75	20 mL/well

PROTOCOL FOR Th1 DIFFERENTIATION *CONTINUED*

8. On day 6 of differentiation, the differentiated mouse Th1 cells are ready to be used for downstream applications.
9. To verify Th1 cell differentiation via flow cytometry, collect the cells and wash with X-VIVO™ 15 Medium once, resuspend the cells in 1 mL 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 4-5 hours. Analyze cytokine expression via flow cytometry.

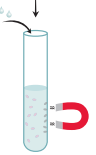
PROTOCOL OUTLINE

Coat the desired tissue culture plate with Hamster Anti-Mouse CD3, Th1 antibody.

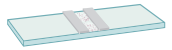


Isolate mouse splenocytes.

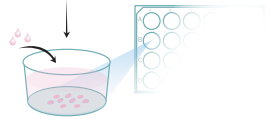
Isolate mouse naïve $CD4^+$ T cells from splenocytes (*e.g.*, using magnetic cell selection).



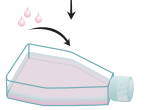
Perform a cell count.



Suspend 1 x 10^6 naïve $CD4^+$ T cells/mL in Mouse Th1 Differentiation Media. Culture the cells on plates pre-coated with Hamster Anti-Mouse CD3 antibody.



Harvest cells on day 3.
Dilute cells 1:10 with fresh Mouse Th1 Differentiation Media.
Culture cells in a new plate or flask for 3 days.



Verify Th1 cell differentiation by analyzing cytokine expression via flow cytometry or ELISA (optional).