DATA EXAMPLES

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

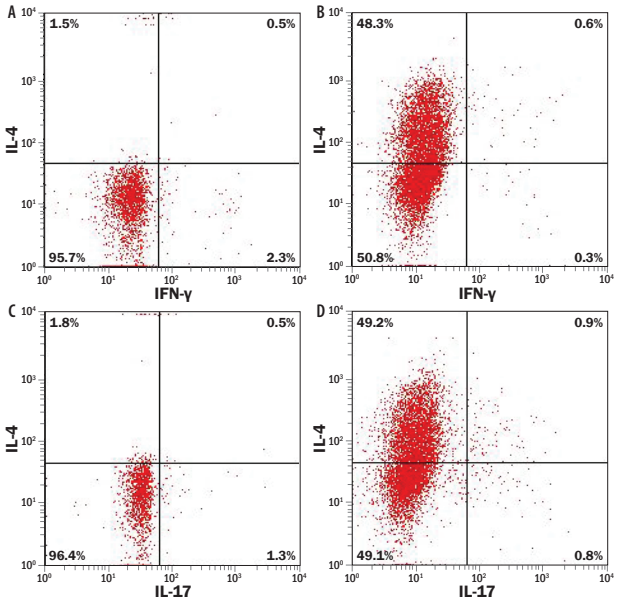

Figure 1: Intracellular Cytokine Staining of Differentiated Mouse Th2 Cells. Flow cytometry data of mouse naïve CD4$^+$ T cells (A, C) and differentiated Th2 cells (B, D) generated using reagents included in this kit. After 6 days of differentiation, naïve CD4$^+$ cells or differentiated Th2 cells were stimulated with Cell Activation Cocktail (Tocris®, Catalog # 5476) and stained with conjugated Anti-Mouse IL-4 (Clone 11B11), Anti-Mouse IFN-γ, 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*

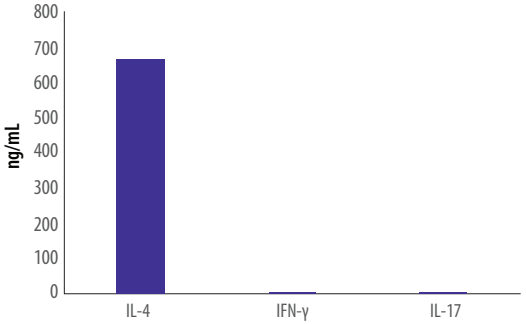

Figure 2: Th2-differentiated Mouse CD4$^+$ Cells Secrete High Levels of IL-4. Mouse naïve CD4$^+$ T cells were differentiated for 6 days using the reagents included in this kit. On day 6 of differentiation cells were harvested and restimulated with Anti-Mouse CD3 and Anti-Mouse CD28 overnight. Cell culture supernatant was collected and cytokine secretion determined using the Mouse IL-4 Quantikine® ELISA Kit, the Mouse IFN-γ 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. Li, Z. *et al.* (2013) Prot. Cell **2**:604.
2. Luckheeram, R.V. *et al.* (2012) Clin. Dev. Immunol. **2012**:925135.
3. Hirahara, K. *et al.* (2011) Immunology **134**:235.3.

CellXVivo™

Mouse Th2 Cell Differentiation Kit

Catalog Number: CDK019

BACKGROUND

CD4$^+$ T cells differentiate into separate T helper cells under the influence of various cytokines and cellular interactions that induce expression of specific transcription factors. T helper type 2 (Th2) cells are a lineage of CD4$^+$ effector T cells that provide host protection against intestinal parasites and extracellular bacteria. In addition, they provide support for B cell-dependent humoral responses. Pathological Th2 cell activity is also a hallmark of allergic inflammation and asthma (1). Differentiation of naïve CD4$^+$ T cells into the Th2 lineage is promoted by cytokines such as IL-4 in combination with either IL-2, IL-7, or TSLP (2, 3). Th2 cells secrete IL-4, IL-5, IL-9, IL-13, and IL17E/IL-25. The CellXVivo™ Mouse Th2 Cell Differentiation Kit contains the necessary components to differentiate mouse naïve CD4$^+$ T cells into Th2-polarized cells that are IL-4$^+$ IFN-γ$^-$ IL-17$^-$. The quantity of the components in the kit is sufficient to differentiate approximately 4x10^6 naïve CD4$^+$ T cells, and generate 80 x 10^6 CD4$^+$ T cells of which > 40% are IL-4$^+$ Th2 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, Th2	968098	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, Th2	968099	1 vial	
Mouse Th2 Reagent 1	968100	1 vial	
Mouse Th2 Reagent 2	968101	1 vial	
Mouse Th2 Reagent 3	968102	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	2 vials	
20X Wash Buffer	967557	3 vials	

* Provided this is within the expiration date of the kit.

OTHER MATERIALS & SUPPLIES REQUIRED

- Laboratory mice
- MagCellect™ Mouse Naive 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 Th2 Reagent 1 (400X) - Add 250 µL of Reconstitution Buffer 1 to Mouse Th2 Reagent 1 to produce Mouse Th2 Reagent 1 (400X).

Mouse Th2 Reagent 2 (400X) - Add 250 µL of Reconstitution Buffer 1 to Mouse Th2 Reagent 2 to produce Mouse Th2 Reagent 2 (400X).

Mouse Th2 Reagent 3 (400X) - Add 250 µL of Reconstitution Buffer 2 to Mouse Th2 Reagent 3 to produce Mouse Th2 Reagent 3 (400X).

Rat Anti-Mouse CD28 (400X) - Add 250 µL of Reconstitution Buffer 2 to Rat Anti-Mouse CD28, Th2, to produce Rat Anti-Mouse CD28 (400X).

Mouse Th2 Differentiation Media - Add Rat Anti-Mouse CD28 (400X), Mouse Th2 Reagent 1 (400X), Mouse Th2 Reagent 2 (400X), and Mouse Th2 Reagent 3 (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 (100X) - Add 150 µL of Reconstitution Buffer 2 to Hamster Anti-Mouse CD3, Th2, to produce Hamster Anti-Mouse CD3 (100X).

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 (100X) stock 1:100 with Wash Buffer (1X) to produce Hamster Anti-Mouse CD3 (1X). Make fresh as needed.

PROTOCOL FOR Th2 DIFFERENTIATION

The quantity of the components in the kit is sufficient to differentiate approximately 4x10^6 naïve CD4+ T cells, and generate 80 x 10^6 CD4+ T cells of which > 40% are IL-4+ Th2 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:

TISSUE CULTURE PLATE	SUGGESTED COATING VOLUME	# OF WELLS PER KIT
24-well plate	0.4 mL/well	Up to 3
96-well plate	0.1 mL/well	Up to 20

   b. Incubate overnight at 2-8 °C or 2-3 hours at 37 °C.
   c. Wash plate twice with Wash Buffer (1X) just prior to adding cells.
2. Prepare a single cell suspension of mouse splenocytes and isolate the 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 1 x 10^6 cells/mL in Mouse Th2 Differentiation Media.
4. Add the cells to a Hamster Anti-Mouse CD3 antibody-coated plate using the suggested volumes below.

TISSUE CULTURE PLATE	SUGGESTED VOLUME
24-well plate	1.3 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 from each well and dilute around 1:20 in fresh Mouse Th2 Differentiation Media in an appropriate sized conical tube.
7. Transfer diluted cells to a new, uncoated flask using the volumes indicated in the table below (ie., 1 well from a 24-well plate can be expanded into 1 T-75 flask or 3 T-25 flasks). Incubate the cells in a 37 °C, 5% CO_2 humidified incubator for 3 days.

FLASK	SUGGESTED VOLUME
T-25	8 mL/flask
T-75	25 mL/flask

8. On day 6 of differentiation, the differentiated mouse Th2 cells are ready to be used for downstream applications.
9. To verify Th2 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 (See Figure 1).

PROTOCOL OUTLINE