FlowCellect™ Mouse T_H1/T_H2 Intracellular Cytokine Kit

25 Tests

Cat. No. FCIM025137

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures.

USA & Canada Phone: +1 (800) 437-7500 Fax: +1 (951) 676-9209
www.millipore.com

Background

There are five main subsets of differentiated CD4+ T-cells; T_H1, T_H2, T_H17, T_{Reg} and T_{FH} cells. Each subtype expresses a signature cytokine or transcription factor that directs the immune response and acts to regulate differentiation.

T_H1 CD4+ T-cells are important for protection against intracellular bacteria, fungi, protozoa and viruses and are involved in some autoimmune responses (1). T_H1 CD4+ T-cells mediate immune responses to intracellular pathogens and respond by producing interferon gamma (IFN-γ). Through secretion of IFN-γ, T_H1 CD4+ T-cells activate macrophages, natural killer (NK) cells and CD8+ T-cells. IFN-γ also increases the production of interleukin-12 by dendritic cells and macrophages, and via positive feedback and stimulates the production of IFN-γ, thereby promoting the T_H1 profile. IFN-γ also inhibits the production of interleukin-4, an important cytokine associated with the T_H2 subtype, and thus it also acts to preserve its own pathway of differentiation.

T_H2 CD4+ T-cells provide protection against extracellular parasites but have also been shown to cause heightened allergic responses to develop (1). They mediate immune responses to extracellular pathogens and respond by producing interleukin 4 (IL-4). Through secretion of IL-4, T_H2 CD4+ T-cells activates B-cells to develop into IgE-secreting cells and thus activating basophil and mast cells to release histamine, a major contributor to allergic responses. IL-4 also increases the production of IL-4 by T_H2 CD4+ T-cells, thereby promoting the T_H2 profile.

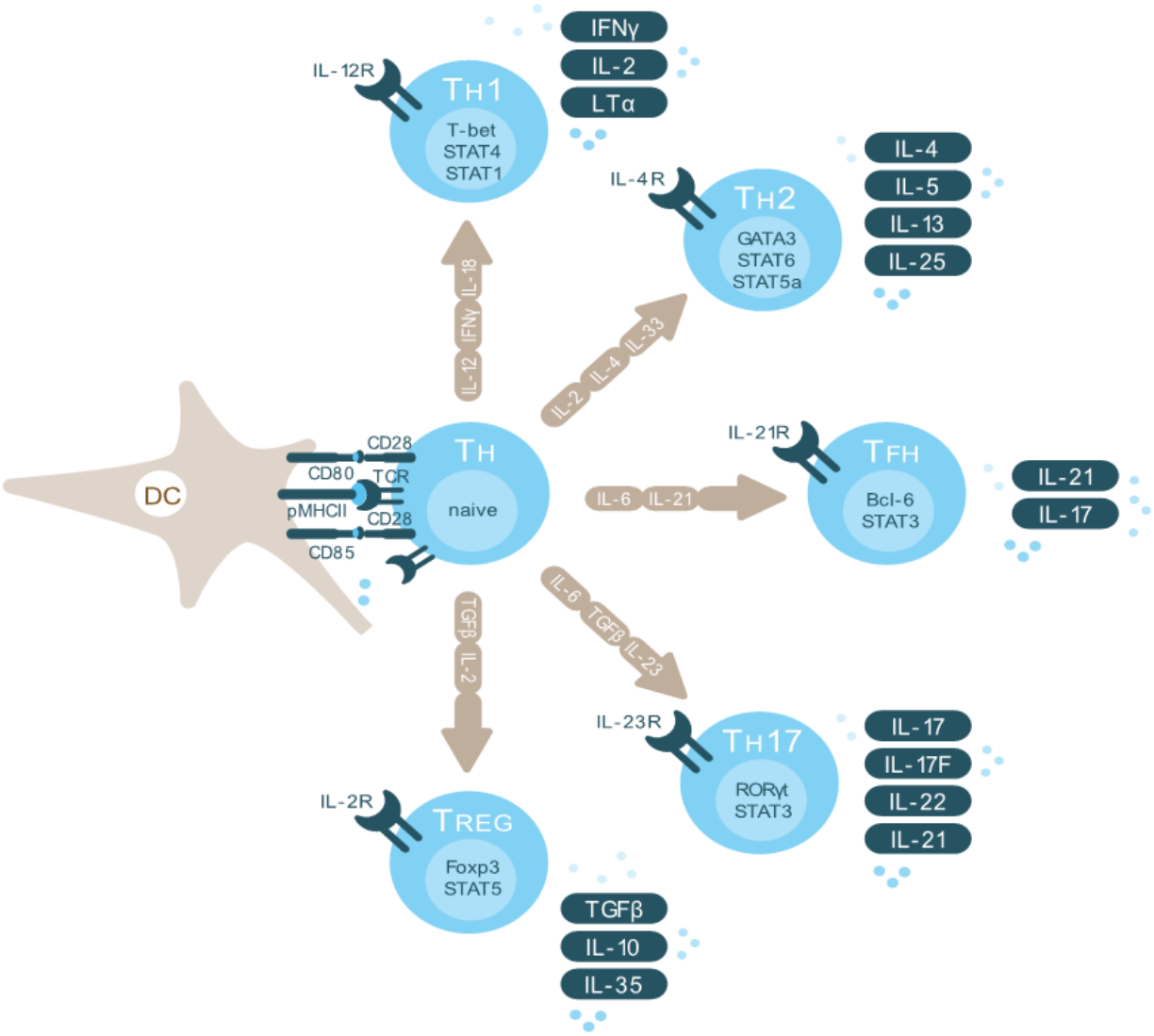

Figure 1: Schematic diagram of CD4+ T-cell differentiation. Five different subtypes of CD4+ T-cells can develop from a common naïve precursor depending on the cytokine environment and interaction with dendritic cells (DC).

Application

Millipore's FlowCellect™ Mouse T_H1/T_H2 Identification Kit is designed to enable a researcher a quick and easy way to detect IFN-γ and IL-4 expression in mouse T_H1 and T_H2 CD4+ T-cells.

Millipore's FlowCellect™ Mouse T_H1/T_H2 Identification Kit contains a PE conjugated antibodies to IFN-γ and IL-4 as well as a PerCP conjugated antibody to CD4. The kit also includes optimized cytokine blocking reagents and buffers to aid in identifying T_H1 and T_H2 CD4+ T-cell subsets in ex vivo lymphocyte populations or to monitor T_H1 and T_H2 CD4+ T-cell differentiation in culture. In addition, Millipore has added a fixable viability dye that eliminates false positive data resulting from cytokine staining on dead cells that can occur in long term cultures. The Millipore's FlowCellect™ Mouse T_H1/T_H2 Identification Kit contains sufficient reagents for 25 3-color tests. Detailed assay instructions are included to assist in analysis and to ensure that the correct cell concentration is obtained during acquisition of sample data. This kit is not designed to be used in the analysis of whole spleen cells in SJL mice.

For Research Use Only; Not for use in diagnostic procedures

Kit Components

FCIM025137-1, FlowCellect™ Mouse T_H1/ T_H2 Intracellular Cytokine Kit, store at 2-8°C

1. 20X Anti-CD4-PerCP clone GK1.5 : (Part No. CS205790) 1 vial containing 150 µL
2. 20X Anti-IL-4-PE, clone 11B11: (Part No.CS206500) 1 vial containing 150 µL
3. 20X Anti-IFNγ-PE, clone XMG1.2: (Part No.CS206353) 1 vial containing 150 µL
4. Fixation Buffer: (Part No. CS202122) One bottle containing 13 mL buffer
5. 10X Wash Buffer: (Part No. CS202123) One bottle containing 13 mL buffer
6. 5X Assay Buffer: (Part No. CS202124) One bottle containing 55 mL buffer
7. Permeabilization Buffer Concentrate: (Part No.CS206346) One bottle containing 15 mL buffer
8. 1000X Monensin Buffer: (Part No.CS206344) One vial containing 50 µL
9. 1000X Brefeldin A Buffer: (Part No.CS206347) One vial containing 50 µL

CF200098, store at -80°C

10. 1000X Fixable Viability Dye eFluor®660: One vial containing 50 µL∗

∗Note: Refer to the Preparation of Buffers and Storage sections of the manual for detailed instructions on Fixable Viability Dye eFluor®660 storage and preparation.

Materials Not Supplied

1. Flow Cytometer with a 488nm and a 633nm laser
2. Mouse splenocytes
3. Pipettors with corresponding tips capable of accurately measuring 1 – 1000 μL
4. Tabletop centrifuge capable of 2500 rpm.
5. Tubes capable of holding 1 mL and 2 μL of sample
6. Red blood cell lysis buffer (Millipore catalog #4700-0088)
7. Activators and stimulants to differentiate T_H1 or T_H2 cells

Precautions

- The instructions provided have been designed to optimize the kit's performance. Deviation from the kit's instructions may result in suboptimal performance and may produce inaccurate data.
- Some assay components included in the kit may be harmful. The kit includes a fixation solution containing formaldehyde. Please refer to the MSDS sheet which can be found at www.millipore.com for specific information on hazardous materials.
- All fluorochrome conjugated antibodies are light sensitive and must be stored in the dark at 2-8°C.
- During storage and shipment, small volumes of product will occasionally become entrapped in the seal of the product vial. For maximum recovery of product, centrifuge vial briefly prior to removing cap.
- Do not use reagents beyond 4 months from date of receipt.

Storage

Caution: The fixable viability dye should be protected from light and moisture and stored at -80°C with desiccant. Upon receipt, all antibodies and buffers should be stored at 2-8°C. *Fluorochrome conjugated antibodies should always be stored at 2-8°C. Do not freeze fluorescent antibodies. Any deviation in temperature for long periods of time may compromise the performance of the antibodies.*

Preparation of Buffers

The 1000X fixable viability dye is sensitive to light, moisture and temperature changes and therefore should be thawed in the dark and stored at -80°C into single use aliquots with a desiccant pack.

- Thaw vial of 1000X Fixable Viability Dye eFluor®660 in the dark. Spin down vial briefly and aliquot 2 μL in 25 single use tubes. Use 1 μL per test.

Prepared 1X Assay Buffer, 1X Wash Buffer and Permeabilization Buffer are stable up to four months if stored at 2-8ºC, and can be prepared when you receive the kit or when you start the first assay.

- Make 100 mL of 1X Assay Buffer: Mix 20 mL of 5X Assay Buffer with 80 mL of deionized water.
- Make 30 mL of 1X Wash Buffer: Mix 3 mL of 10X Wash Buffer with 27 mL of deionized water.
- Make 30 mL of Permeabilization(Perm) Buffer/10X10^6 cells: Mix 6 mL of 5X Assay Buffer with 0.3 mL of Permeabilization Buffer Concentrate and 24 mL of deionized water.

Prepared Permeabilization/Fixation Buffer must be made fresh each time the assay is performed.

- Make 1 mL of Fixation/Permeabilization(Fix/Perm) Buffer/test: Mix 0.5 mL of Fixation Buffer with 0.05 mL of Permeabilization Buffer Concentrate and 0.45 mL of 1X Assay Buffer.

Cell Staining Protocol

Note: Before starting protocol prepare all buffers and place on ice or store at 2-8ºC.

1. Add 1000X Brefeldin A Buffer and 1000X Monensin Buffer to cells of interest 5 hours before staining. **Note:** Brefeldin A and Monensin can be added to cells during stimulation.
2. Count cells and place 5X 10^6 cells into a sample tube.
3. Spin down cells at 2500 rpm (600Xg) for 3 minutes and discard buffer.
4. Resuspend the cells in 1 mL of 1X Wash Buffer by gently pipetting up and down.
5. Spin down cells at 2500 rpm (600Xg) for 3 minutes and discard buffer.
6. Resuspend the cells in 1 mL of 1X Wash Buffer by gently pipetting up and down. Place cells on ice.

7. Thaw an aliquot of 1000X Fixable Viability Dye eFluor®660 just before use and keep it in the dark at all times. Add 1 μL of 1000X Fixable Viability Dye for every 5 X 10^6 cells. Immediately mix cells and dye gently by pipetting up and down. ***Note****: Once cells are stained with dye keep them away from light as much as possible.*

8. Incubate cells on ice for 15 minutes in the dark.

9. Spin down cells at 2500 rpm (600Xg) for 3 minutes and discard buffer.

10. Resuspend the cells in 1mL of 1X Assay Buffer/5X 10^6 cells by gently pipetting up and down.

11. Repeat steps 9 and 10 once then go to step 12.

12. Resuspend cells in 0.5 mL Permeabilization/Fixation Buffer/5X 10^6 cells by gently pipetting up and down.

13. Incubate cells at room temperature for 20 minutes in the dark.

14. Add 1 mL of 1X Perm Buffer/5X 10^6 cells.

15. Spin down cells at 2500 rpm (600Xg) for 3 minutes and discard buffer.

16. Repeat steps 14 and 15 once then go to step 17.

17. Resuspend cells in 1 mL of Permeabilization Buffer/5X 10^6 cells by gently pipetting up and down. ***Note:*** *At this point the cells can be stored overnight at 4 degrees in the dark.*

18. Mix cells thoroughly and place 0.1 mL of cells in a 1.5 mL tube or 96-well plate.

19. Add 5 μL of 20X Anti-CD4-PerCP and 5 μL of 20X Anti-IL-4-PE or 5 μL of 20X Anti-IFNγ-PE to each tube or well.

20. Incubate cells in the dark and on ice for 1 hour.

21. Add 0.1 mL of Permeabilization Buffer to cells.

22. Spin down cells at 2500 rpm (600Xg) for 3 minutes and discard buffer.

23. Resuspend cells in 0.2 mL of Permeabilization Buffer by gently pipetting up and down.

24. Spin down cells at 2500 rpm (600Xg) for 3 minutes and discard buffer.

25. Resuspend cells in 0.2 mL of 1X Assay Buffer by gently pipetting up and down.

26. Dilute cells in 1X Assay Buffer to an appropriate concentration for flow cytometer to be used and analyze.

Sample Data

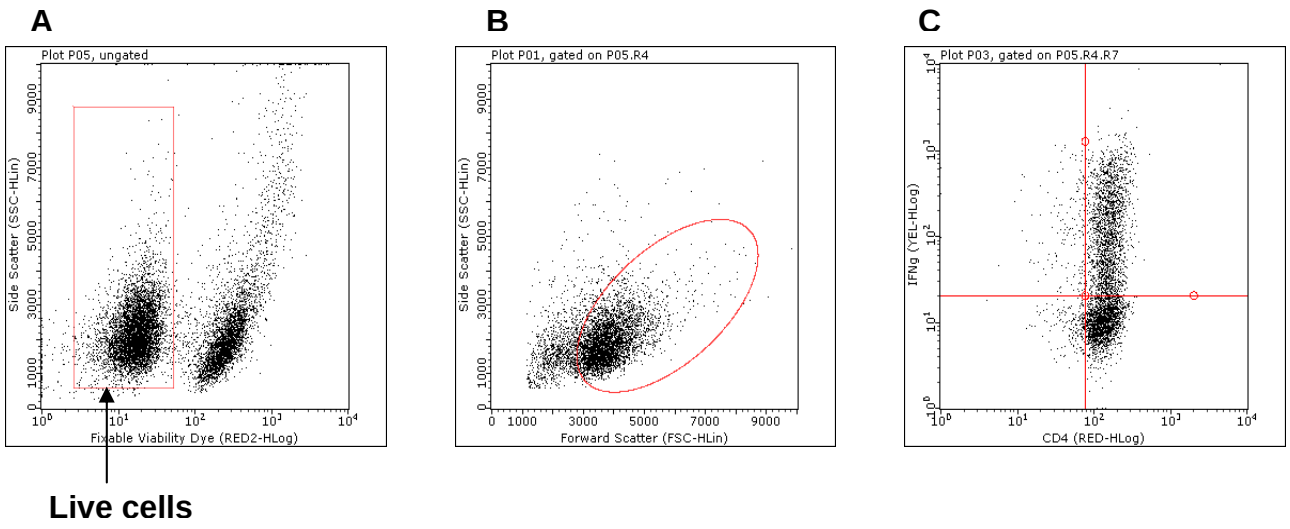

Figure 2: Representative flow cytometric data of T_H1 differentiated CD4+ mouse T-cells stained with Fixable Viability Dye eFluor®660, anti-CD4-PerCP and anti-IFNγ-PE. Dot plot of ungated cells stained with fixable viability dye (A). Forward and side scatter dot plot of live gated cells from plot A (B). Differentiated T_H1 CD4+ T-cells gated on lymphocyte population in plot B and stained with anti-CD4-PerCP and anti-IFNγ-PE(C).

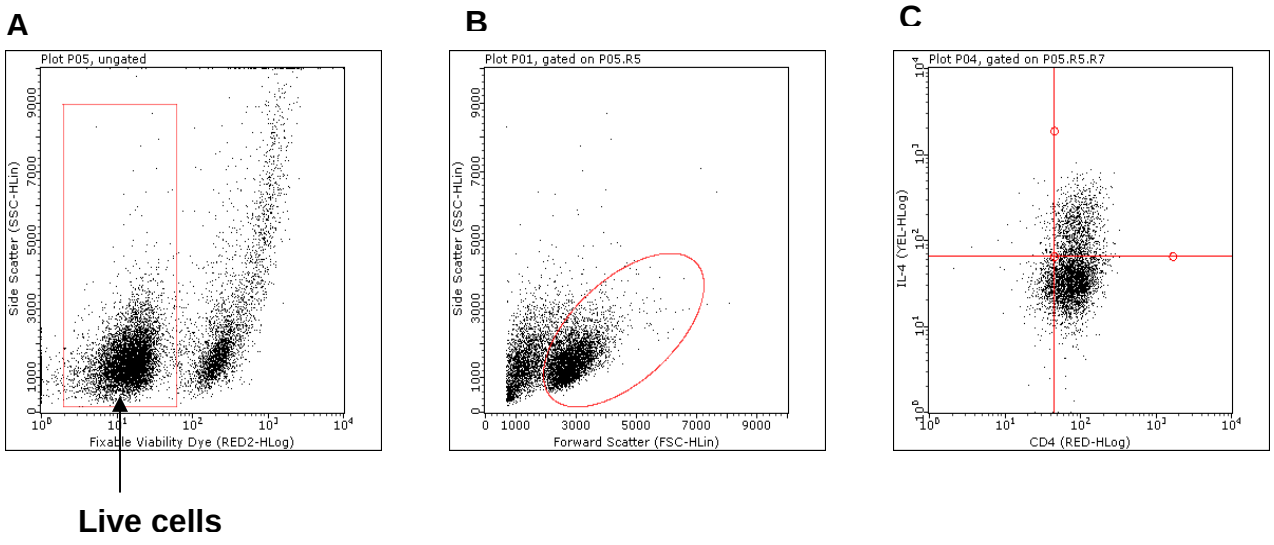

Figure 3: Representative flow cytometric data of T_H2 differentiated CD4+ mouse T-cells stained with Fixable Viability Dye eFluor®660, anti-CD4-PerCP and anti-IL-4-PE. Dot plot of ungated cells stained with fixable viability dye (A). Forward and side scatter dot plot of live gated cells from plot A (B). Differentiated T_H2 CD4+ T-cells gated on lymphocyte population in plot B and stained with anti-CD4-PerCP and anti-IL-4-PE(C).

Technical Hints

- The Permeabilization Buffer concentrate may have crystallization or precipitation when it is stored at 2-8°C, however, it's normal and does not affect the buffer performance. *Do not heat, vortex or filter.* If crystals form, avoid them when taking out an aliquot.
- If minor precipitate is detected in the 10X Wash Buffer place the bottle in a warm water bath for 30 minutes, followed by mixing the contents on a mechanical vortex.
- For cellular staining and analysis to be most effective, make sure that test cells have good viability prior to use.
- Certain cell cultures cell pellets may become hazy or transparent following the fixation step, making it difficult to see. After the washing steps they will become easier to visualize.
- If sampling a small collection of cells for flow analysis, it is recommended that all steps be performed in a smaller collection tube (e.g. centrifuge tube)
- Do not mix or interchange reagents from various kit lots.

Troubleshooting

Potential Problem	Experimental Suggestions
Acquisition rate decreases dramatically Instrument clogging Too many cells	• Cell concentration too high - Decrease the number of cells per microliter by diluting sample to 300 – 500 cells per microliter. Guava cytometers give the most accurate data when the flow rate is less 500 cells per microliter. • Run three Quick Cleans (for Guava cytometers) to rinse out the flow cell. This procedure can be performed during or after an assay. This will wash away any material forming within the glass capillary walls.
Too few cells	• Spin down cells and resuspend in a smaller volume. The assay instructions are optimized to give you a range of cells between 100-500 cells/μL in the final sample volume. However, cell loss is common during washing steps in the assay procedure. A substantial decrease in cell numbers can lead to difficulty in adjusting settings. Make sure to leave the cell pellet intact when discarding buffer. If the cells are not generating a compact pellet after centrifugation, increase the time to 5 minutes and/or increase the speed by 500 rpm until a compact and visible cell pellet forms.
Background staining and/or non-specific staining of cells	• This assay was optimized using mouse spleen cells. Therefore, further antibody titrations may be necessary for other cell types and conditions to capture the ideal staining concentration. Non-specific staining and background may indicate that less antibody will need to be used during the staining procedure.
Variability in day to day experiments	• Monitor cell viability and cell numbers being analyzed are consistent. Any changes in culture conditions or viability can influence experimental results. •Make sure that a quality check on the flow cytometer to be used (e.g. calibration) is performed on a daily basis prior to use.

For further support, please contact Millipore's Technical services at +1(800) 437-7500

References

1. Wan YY. Multitasking of helper T cells. Immunology 2010 Jun;130 (2):166-71.

Related Products

1. FlowCellect™ Mouse FoxP3 Treg Identification Kit (Catalog No. FCIM025126)
2. FlowCellect™ Human Lymphocyte ZAP-70 Characterization Kit (Catalog No. FCIM025122)

Warranty

Millipore Corporation (“Millipore”) warrants its products will meet their applicable published specifications when used in accordance with their applicable instructions for a period of one year from shipment of the products. **MILLIPORE MAKES NO OTHER WARRANTY, EXPRESSED OR IMPLIED. THERE IS NO WARRANTY OF MERCHANTABILITY OR FITNESS FOR A PARTICULAR PURPOSE.** The warranty provided herein and the data, specifications and descriptions of Millipore products appearing in Millipore’s published catalogues and product literature may not be altered except by express written agreement signed by an officer of Millipore. Representations, oral or written, which are inconsistent with this warranty or such publications are not authorized and if given, should not be relied upon.

In the event of a breach of the foregoing warranty, Millipore’s sole obligation shall be to repair or replace, at its option, the applicable product or part thereof, provided the customer notifies Millipore promptly of any such breach. If after exercising reasonable efforts, Millipore is unable to repair or replace the product or part, then Millipore shall refund to the Company all monies paid for such applicable Product. **MILLIPORE SHALL NOT BE LIABLE FOR CONSEQUENTIAL, INCIDENTAL, SPECIAL OR ANY OTHER DAMAGES RESULTING FROM ECONOMIC LOSS OR PROPERTY DAMAGE SUSTAINED BY ANY COMPANY CUSTOMER FROM THE USE OF ITS PRODUCTS.**

Unless otherwise stated in our catalog or other company documentation accompanying the product(s), our products are intended for research use only and are not to be used for any other purpose, which includes but is not limited to, unauthorized commercial uses, in vitro diagnostic uses, ex vivo or in vivo therapeutic uses or any type of consumption or application to humans or animals.

Cat No. FCIM025137

09/2010
Revision A: FCIM025138MAN