Mouse Th1/Th2 Flow Cytometry Staining Kit

Cat. No: XJM001 **Size: 20 Assays/100 Assays**

Kit Components

Cat.	Products	20 Assays	100 Assays	Storage
XJM001A	Mouse Th1/Th2 Cytokine Detection Antibody Cocktail	200 µL	1 mL	2~8°C, shading light
XJM001B	Mouse Th1/Th2 Cytokine Detection Antibody Isotype Cocktail	200 µL	1 mL	2~8°C, shading light
E-CK-A011	Cell Stimulation MIX Powder (50 µg)	50 µg	50 µg×3	-20℃/-80℃, shading light
E-CK-A012	Cell Stimulation MIX Solvent	120 µL	120 µL×3	-20°C, shading light
E-CK-A013	Protein Transport Inhibitor MIX Powder (200 µg)	200 µg	200 µg×3	-20℃/-80℃, shading light
E-CK-A109A	Fixation Buffer	10 mL	10 mL	2~8°C
E-CK-A109B	Permeabilization Buffer (5×)	15 mL	50 mL	2~8°C
	Manual		One Copy	

Composition of Components

Products	Component
Mouse Th1/Th2 Cytokine Detection Antibody Cocktail	PerCP/Cyanine5.5 Anti-Mouse CD3 Antibody[17A2]
	FITC Anti-Mouse CD4 Antibody[RM4-5]
	PE Anti-Mouse IFN-γ Antibody[XMG1.2]
	APC Anti-Mouse IL-4 Antibody[11B11]
Mouse Th1/Th2 Cytokine Detection Antibody Isotype Cocktail	PerCP/Cyanine5.5 Anti-Mouse CD3 Antibody[17A2]
	FITC Anti-Mouse CD4 Antibody[RM4-5]
	PE Rat IgG1,κ Isotype Control[HRPN]
	APC Rat IgG1,κ Isotype Control[HRPN]

Note: It is not recommended to mix Cocktail from different batches of kits.

Storage

1. The reagents in this kit can be stored for 12 months under the recommended conditions.
2. Cell Stimulation MIX powder and Protein Transport Inhibitor MIX powder can be stored at -20°C and with shading light for 1 year, and at -80°C with shading light for 2 years. The dry powders can be dissolved and stored at -20°C and protected from light for 6 months, or can be dispensed and stored at -80°C and protected from light for 1 year.

Introduction

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The Elabscience® Mouse Th1/Th2 Flow Cytometry Staining Kit can be used to detect the proportion of Th1 and Th2 cells among T lymphocytes (or $CD4^+$ helper T cells) in mouse splenocytes and fresh mouse anticoagulated blood. Th cells, also known as helper T cells (helper T cells), have a very weakly ability to differentiate from Th0 to Th1, Th2, and Th17 cells in the resting state (unstimulated state, e.g. normal physiological state). There are only a very small number of Th1, Th2 and Th17 cells in the peripheral blood, when the Th cells are stimulated by external factors (e.g, stimuli, pathogens, etc.), the Th0 will differentiate into Th1, Th2 or Th17, and the specific tendency of the differentiation will depend on the type of cytokine in the microenvironment. The proportion of Th1, Th2 or Th17 cells in the activated T lymphocytes can be known by detecting the expression of IFN-γ, IL-4 or IL-17A in the sample. This product provides a mixture of four-color fluorescently labeled antibodies that specifically bind to mouse CD3, CD4, IFN-γ (for detection of Th1), and IL-4 (for detection of Th2), allowing for the detection of Th1 and Th2 cells by multicolor panel.

Activated cells are stimulated to secrete cytokines by Cell Stimulation MIX in the kit, and the induced secretion of cytokines is further blocked intracellularly by the Protein Transport Inhibitor MIX. The activated T cells were fixed by Fixation Buffer and then permeabilized with Permeabilization Buffer to fully permeabilize the cell membrane, and the permeabilization could be synchronized with the binding of cell surface/intracellular antigens and antibodies, and finally the intracellular secretion of cytokines from the activated T cells was detected by flow cytometry.The surface markers of the Th cells are $CD3^+CD4^+$, and the correct gate was selected for $CD4^+$ T cells, and the proportion of Th1 and Th2 cells in the activated T lymphocytes was further analyzed by detecting different fluorescence signals on IFN-γ and IL-4 antibodies.

Materials Not Supplied

- **Reagents**

RPMI-1640, L-alanyl-L-glutamine solution (200 mM), penicillin-streptomycin solution, fetal bovine serum, cell staining buffer (E-CK-A107) or 1× PBS, DMSO, 10× ACK lysis buffer (E-CK-A105), deionized water.

- **Instruments**

Flow cytometer, CO_2 incubator, centrifuge.

Reagent Preparation

1) 500× Cell Stimulation MIX

Add 100 μL Cell Stimulation MIX Solvent to dissolve a vial of Cell Stimulation MIX Powder (50 μg) and mix fully, and store at -20℃ away from light.

Note: Centrifuge at 2000~10000×g for several seconds before use and then open the cover for use.

2) 1000× Protein Transport Inhibitor MIX

Add 50 μL of pre-cooled 33% DMSO solution (supplied) to each tube of Protein Transport Inhibitor MIX Powder (200 μg) and mix well to form 1000× Protein Transport Inhibitor MIX, store at -20℃ away from light.

Note: Centrifuge at 2000~10000×g for several seconds before use and then open the cover for use. 33% DMSO solution can be prepared by mixing 670 μL of sterile ultrapure water or sterile deionized water with 330 uL of DMSO solution, and can be stored at -20℃ away from light after preparation.

3) 1× Permeabilization Buffer

Dilute Permeabilization Buffer (5×) with deionized water to 1×Permeabilization Working Solution before use.

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For example, take 1 mL Permeabilization Buffer (5×) to 4 mL deionized water and mix fully to prepare 1× Permeabilization Working Solution.

Note: It is recommended that 1×Permeabilization Working Solution be used up within 3 days.

4) RPMI-1640 complete medium

Add L-alanyl-L-glutamine solution (final concentration of 2 mM), penicillin solution (final concentration of 100 U/mL), streptomycin solution (final concentration of 100 U/mL), and fetal bovine serum (final concentration of 10%) to RPMI 1640 medium. The prepared medium can be stored at 4℃ for two weeks.

Experimental Protocol

➢ **Mouse spleen cell samples**

Note: Sterile technique is required for the step 1-5.

1. Take fresh mouse spleen samples and prepare into single-cell suspension, centrifuge at 300×g for 5 min, and discard the supernatant.
2. Add 2 mL of 1×ACK Lysis Buffer (E-CK-A105, self-prepared) to resuspend the cells, and lysis for 2 min at room temperature.
   Note: The lysis time should not exceed 2 min to prevent excessive lysis. When the ambient temperature exceeds 25°C, lysis operation can be carried out at 4°C to maintain the cell's state.
3. Immediately add 12 mL of PBS buffer or RPMI-1640 basal medium to terminate lysis, centrifuge at 300×g for 5 min, and discard the supernatant.
4. Add an appropriate amount of RPMI-1640 complete medium to resuspend the cells and count the cells. Adjust the cell density to 1×10^6 cells/mL with complete medium and seeded.
5. Add Cell Stimulation MIX and Protein Transport Inhibitor MIX with reference to the table below, incubate at 37°C, 5% CO_2 incubator for 5 h, and mix every 1-2 h.

Group	Experimental Content	Addition of Antibodies
Negative group	Untreated	XJM001A
Test group	Add 2 μL of Cell Stimulation MIX (500×) and 1 μL of Protein Transport Inhibitor MIX (1000×) to each 1 mL cell suspension	
Isotype group		XJM001B

In the subsequent steps, sterile technique is not necessary.

6. Collect the cells, centrifuge at 300×g for 5 min, discard the supernatant, add 1 mL Cell Staining Buffer or 1×PBS and resuspend the cells, centrifuge at 300×g for 5 min, discard the supernatant.
7. Each 1×10^6 cells were resuspended with 180 μL of Cell Staining Buffer or 1×PBS, then add 60 μL of Fixation Buffer and mix thoroughly, then fix the cells overnight at 4°C or at room temperature for 1 h.
8. The fixed cells will settle to the bottom of the EP tube after fixation, discard the supernatant. Add 1~2 mL of 1× Permeabilization Buffer, mix fully and centrifuge at 500×g for 5 min, and discard the supernatant.
9. Add 100 μL of 1× Permeabilization Buffer to resuspend the cells, add 10 μL of antibody according to the above table, and incubate for 60 min at room temperature with shading light, and mix every 15~20 min.
10. After incubation, add 1~2 mL of Cell Staining Buffer or 1×PBS, centrifuge at 500×g for 5 min, and discard supernatant.
11. Add 100~200 μL Cell Staining Buffer or 1×PBS to resuspend the cells, and detect the cytokine expression with

flow cytometry.

Detection indicators	Fluorophore	Ex (nm)	Em (nm)
CD3	PerCP/Cyanine5.5	440, 480, 675	675
CD4	FITC	490	530
IFN-γ	PE	495, 565	575
IL-4	APC	650	660

➢ **Mouse whole blood samples**

Note: Sterile technique is required for the step 1-2.

1. Take fresh mouse whole blood sample anticoagulated with sodium heparin, add 250 µL of anticoagulated blood and 250 µL of RPMI-1640 basal medium (without serum) to each group and mix fully.
2. Add Cell Stimulation MIX and Protein Transport Inhibitor MIX according to the table below, incubate at 37°C, 5% CO_2 incubator for 5 h, and mix every 1-2 h.

Group	Experimental Content	Addition of Antibodies
Negative group	Untreated	XJM001A
Test group	Add 1 µL Cell Stimulation MIX (500×) and 0.5 µL Protein Transport Inhibitor MIX (1000×)	
Isotype group		XJM001B

In the subsequent steps, sterile technique is not necessary.

3. Transfer the cells to a 2 mL EP tube, add 1 mL of Cell Staining Buffer or 1×PBS and mix fully, centrifuge at 300×g for 5 min, and discard the supernatant.
4. Add 100 µL of Cell Staining Buffer or 1×PBS to resuspend the cells, then add 2 mL of 1×ACK Lysis Buffer (E-CK-A105), and lysis on ice for 2~3 min. centrifuge at 300 g for 5 min.
   Note: Lysis is complete when the solution turns from turbid to translucent, please centrifuge in time to avoid excessive lysis and cell damage.
5. Add 1mL Cell Staining Buffer or 1×PBS to resuspend the cells, centrifuge at 300×g for 5 min, and discard the supernatant.
6. Add 180 µL of Cell Staining Buffer or 1×PBS to resuspend the cells, add 60 µL of Fixation buffer and mix thoroughly, and fix the cells at 4°C overnight or at room temperature for 1 h.
7. After fixation, the cells will settle to the bottom of the EP tube, discard the supernatant. Add 1~2 mL of 1× Permeabilization Buffer, centrifuge at 500×g for 5 min, and discard the supernatant.
8. Add 100 µL of 1× Permeabilization Buffer to resuspend the cells, add 10 µL of antibody according to the above table, and incubate for 60 min at room temperature with shading light, and mix every 15~20 min.
9. After incubation, add 1 mL of Cell Staining Buffer or 1×PBS, mix fully, centrifuge at 500×g for 5 min, and discard the supernatant.
10. Add 100~200 µL Cell Staining Buffer or 1×PBS to resuspend the cells, and detect the cytokine expression with flow cytometry.

Detection indicators	Fluorophore	Ex (nm)	Em (nm)
CD3	PerCP/Cyanine5.5	440, 480, 675	675
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IL-4	APC	650	660

Cautions

1. This product is for scientific use only.
2. Permeabilization Buffer (5×) may cause precipitation, which is normal and does not affect the effectiveness.
3. When preparing mouse whole blood samples, it is recommended to use sodium heparin anticoagulant, EDTA or similar calcium-chelating anticoagulants may affect cytokine secretion.
4. Due to the different proportion of red blood cells, please pay attention to the lysis time of red blood cells. By observing the solution changing from red turbid to red clear, lysis can be terminated to avoid excessive lysis affecting the cell state.
5. the centrifuge speed up and down too high will cause cell loss, it is recommended to adjust the speed up not more than 3, down not more than 2, that is, Acc ≦3, Dec ≦2.
6. Fluorescent substances are prone to quenching, so be careful to keep them away from light during operation and storage.
7. For your safety and health, please wear laboratory overalls and disposable gloves for operation.

Common Problems and Solutions

Common Problem	Possible Cause	Suggestion
No cytokine expression detected	Excessive cell density.	Adjust cell density to 1~2×10^6 cells/mL.
	Interference from red blood cells.	The lysis time of red blood cells is about 2 min, need to be fully lysed but do not excessively lysed.
	Reagents expired.	Store reagents properly and use them within expiration date.
	Poor cell fixation/permeabilization.	Control fixation/permeabilization time according to the instructions.
	Insufficient induction time.	Conduct a preliminary experiment by settinggradient induction time to explore the optimal reaction time.
Excessive intracellular cytokine expression	Poor cell condition, high number of dead cells.	Ensure cells are in good condition prior to induction and exclude dead cells.
	Non-specific antibody binding.	Increase antibody blocking steps to reduce non-specific staining.
Weak fluorescence signal in flow cytometry	Insufficient amount of antibody used.	Add the antibody according to the instructions.

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	Insufficient antibody staining time.	Extend antibody incubation time according to the instructions or as needed.
	Too many cells.	Reduce cell density.
	Very low target protein expression level.	Enrich target cells before induction and detection.

Typical results

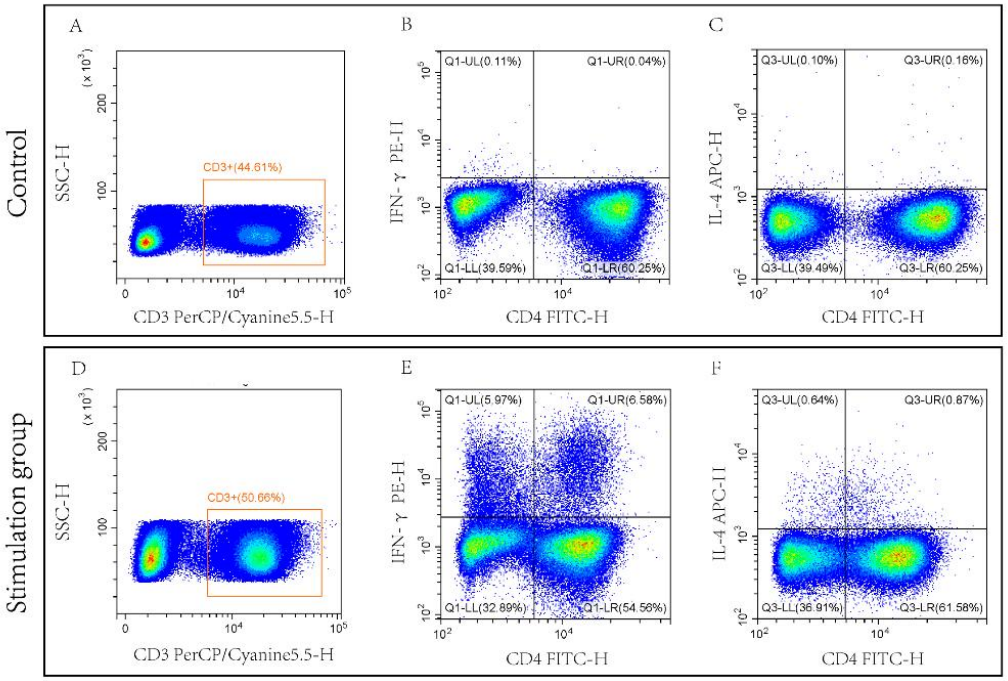


Figure 1: Flow cytometric analysis of Mouse Th1/Th2 Flow Cytometry Staining Kit.Splenocytes from normal C57 mouse (Control) and C57 mouse stimulated with Cell Stimulation MIX (Stimulation group), $CD3^+$ $CD4^+$ IFN-γ^+ cells (Q1-UR)were gated to analyze Th1 and $CD3^+$ $CD4^+$ IL-4^+ cells (Q3-UR)were gated to analyze Th2.

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