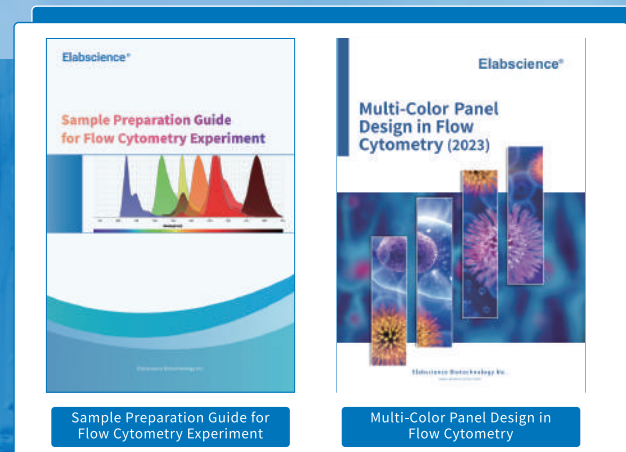


# Phenotyping of Immune Cells by Flow Cytometer (I)

- Phenotyping of Common Immune Cells
- Staining Process of Flow Cytometry
- Elabscience® Featured Flow Cytometry Services

Elabscience®



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# About Elabscience®



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Elabscience® major products cover FCM Antibodies, ELISA Kits, Cell Function Detection Kits, Metabolism Assay Kits, Labeling Kits, Antibodies, Recombinant Proteins, and Immunology Related Reagents.

Elabscience® also offers custom services for our customers including Protein Service, Polyclonal Antibody Service, Peptide Service and Gene Synthesis.

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# Phenotyping of Common Immune Cells

## T/B/NK Cell Population Detection

Lymphocytes (T/B/NK) are important cellular components in the immune response function of the body, usually divided into T lymphocytes, B lymphocytes, and natural killer (NK) cells. The state of the body's immune function can be evaluated based on the changes in T/B/NK cell content.

Cell	Function
T lymphocytes	<ul style="list-style-type: none"> <li>Participate in cellular immunity and directly kill pathogen cells.</li> <li>Release some lymphatic factors to enhance the immune response of the body.</li> </ul>
B lymphocytes	<ul style="list-style-type: none"> <li>Participate in humoral immunity and produce antibodies.</li> <li>Present soluble antigens.</li> <li>Produce cytokines involved in immune regulation.</li> </ul>
NK cells	<ul style="list-style-type: none"> <li>Killing target cells such as viral infections or tumors.</li> <li>Secreting cytokines participates in immune regulation.</li> </ul>

Table 1: Function of different lymphocytes

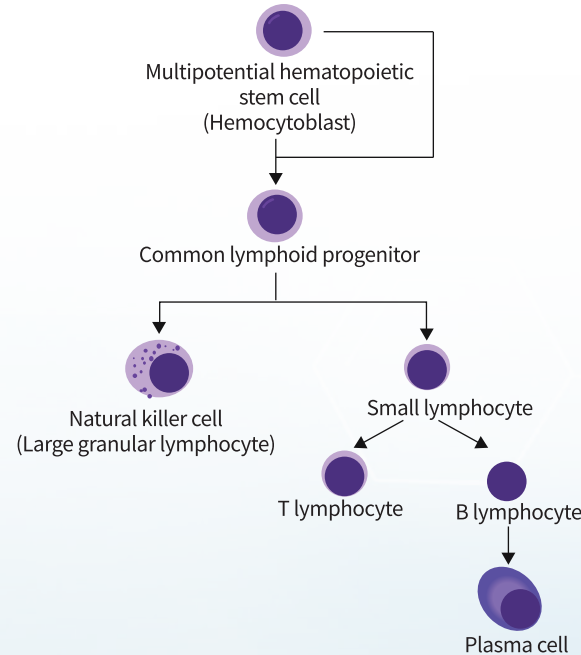


Fig 1: Differentiation of lymphocytes

(Image from Medical Immunology)

## Detection of T/B/NK (4-color) in Human Peripheral Blood

### 01 Panel Design

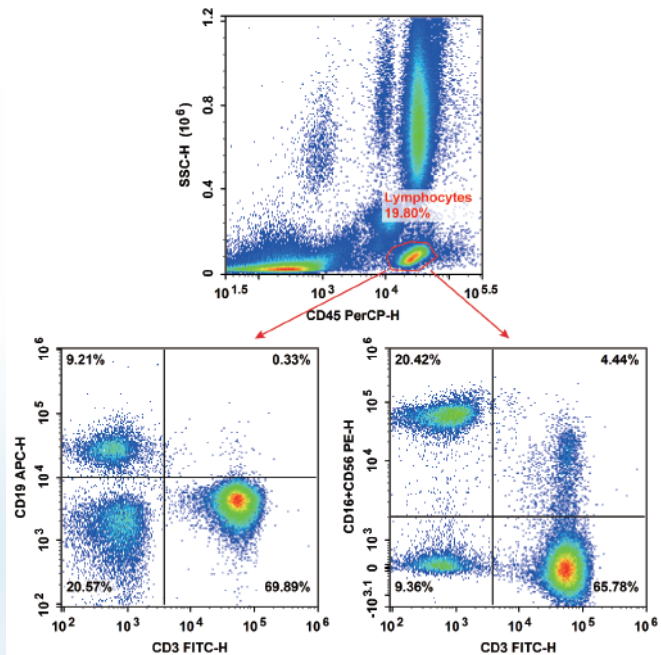
Purpose	Sample	Antibody Collocation
Adjust the voltage	1	Blank
	2	CD45-PerCP
Adjust compensation	3	CD3-FITC
	4	CD16-PE, CD56-PE
	5	CD19-APC
PE-FMO in combination with Isotype Control for auxiliary gating	6	CD45-PerCP, CD3-FITC, CD19-APC; Mouse IgG1, κ Isotype Control-PE
Full Panel	7	CD45-PerCP, CD3-FITC, CD16-PE, CD56-PE, CD19-APC

### 02 Information of Flow Cytometry Antibodies

Marker	Fluorochrome	Clone No.	Cat. No.
CD45	PerCP	HI30	E-AB-F1137F
CD3	FITC	OKT3	E-AB-F1001C
CD16	PE	3G8	E-AB-F1236D
CD56	PE	5.1H11	E-AB-F1239D
CD19	APC	CB19	E-AB-F1004E
Mouse IgG1, κ Isotype Control	PE	MOPC-21	E-AB-F09792D



## Detection of T/B/NK (4-color) in C57BL/6 Mouse Peripheral Blood



### Tips:

- ⊞ This panel has obvious cell populations, and compensation regulation can be performed without single positive tube. But for beginners of Flow Cytometry, it is recommended to set up a single positive tube to adjust compensation.
- ⊞ The detection standard for NK cells is CD3-CD16<sup>+</sup>CD56<sup>+</sup>.
- ⊞ In this panel, it is recommended to set Isotype Control for CD16 and CD56, while other indicators can be omitted due to obvious populations.
- ⊞ It is recommended to stain human peripheral blood samples with CD45, which is beneficial for the lymphocyte phylum gating through CD45 and SSC. It is recommended to use the single positive tube of CD45 to start the machine at low speed and set the threshold.

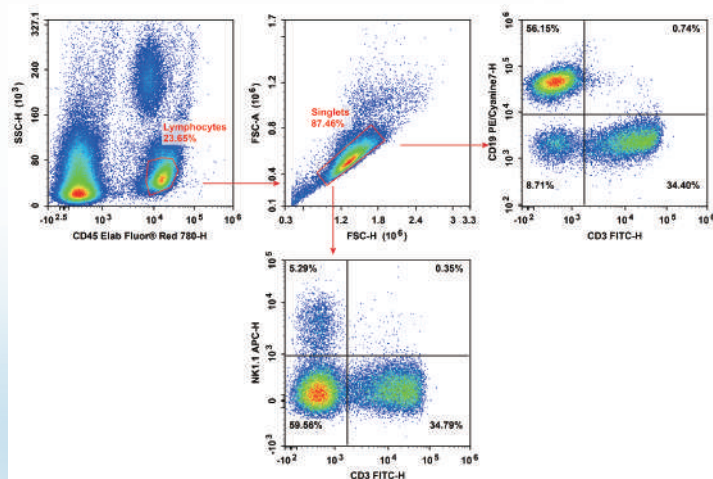
### 01 Panel Design

Purpose	Sample	Antibody Collocation
Adjust the voltage	1	Blank
	2	CD45-Elab Fluor® Red 780
Adjust compensation	3	CD3-FITC
	4	CD19-PE/Cyanine7
	5	NK1.1-APC
APC-FMO in combination with Isotype Control for auxiliary gating	6	CD45-Elab Fluor® Red 780, CD3-FITC, CD19-PE/Cyanine7; Mouse IgG2a, κ Isotype Control-APC
Full Panel	7	CD45-Elab Fluor® Red 780, CD3-FITC, CD19-PE/Cyanine7, NK1.1-APC

### 02 Information of Flow Cytometry Antibodies

Marker	Fluorochrome	Clone No.	Cat. No.
CD45	Elab Fluor® Red 780	30-F11	E-AB-F1136S
CD3	FITC	17A2	E-AB-F1013C
CD19	PE/Cyanine7	1D3	E-AB-F0986H
NK1.1	APC	PK136	E-AB-F0987E
Mouse IgG2a, κ Isotype Control	APC	C1.18.4	E-AB-F09802E





**Tips:**

- ⊞ Add CD45 indicators to peripheral blood samples, the lymphocyte populations can be gated directly through CD45 and SSC.
- ⊞ The CD3/CD4/CD8 cell populations are obvious, it can effectively distinguish between positive and negative cells even without Isotype Control.
- ⊞ The detection indicators of NK cells should be selected based on different mouse varieties, usually C57BL/6 mouse use NK1.1, and BALB/c mouse use CD49b (DX5). CD3-NK1.1<sup>+</sup>/CD3-CD49b<sup>+</sup> is NK cells.
- ⊞ The key factor in this experiment is red blood cell lysis. Excessive or insufficient lysis of red blood cells can lead to unclear lymphocyte grouping.

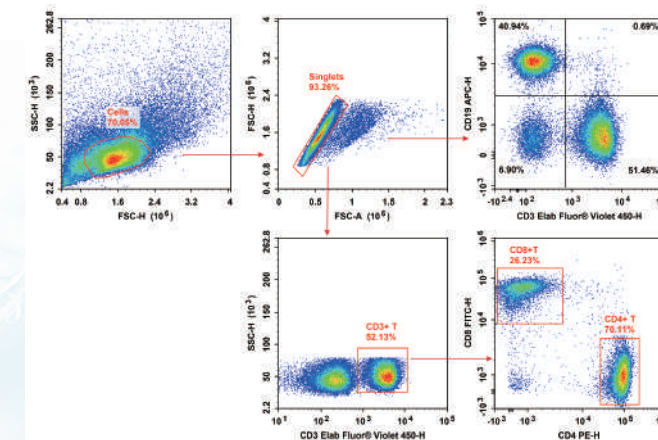
**Detection of TB cells (4-color) in Mouse Lymph Node**

**01 Panel Design**

Purpose	Sample	Antibody Collocation
Adjust the voltage	1	Blank
Full Panel	2	CD3-Elab Fluor® Violet 450, CD4-PE, CD8a-FITC, CD19-APC

**02 Information of Flow Cytometry Antibodies**

Marker	Fluorochrome	Clone No.	Cat. No.
CD3	Elab Fluor® Violet 450	17A2	E-AB-F1013Q
CD4	PE	GK1.5	E-AB-F1097D
CD8a	FITC	53-6.7	E-AB-F1104C
CD19	APC	1D3	E-AB-F0986E



**Tips:**

- ⊞ The lymph nodes are mainly composed of lymphocytes, so there is no need for CD45 Marker.
- ⊞ CD3/CD4/CD8/CD19 cells are easy distinguished, Single Positive, FMO and Isotype Control are unnecessary.

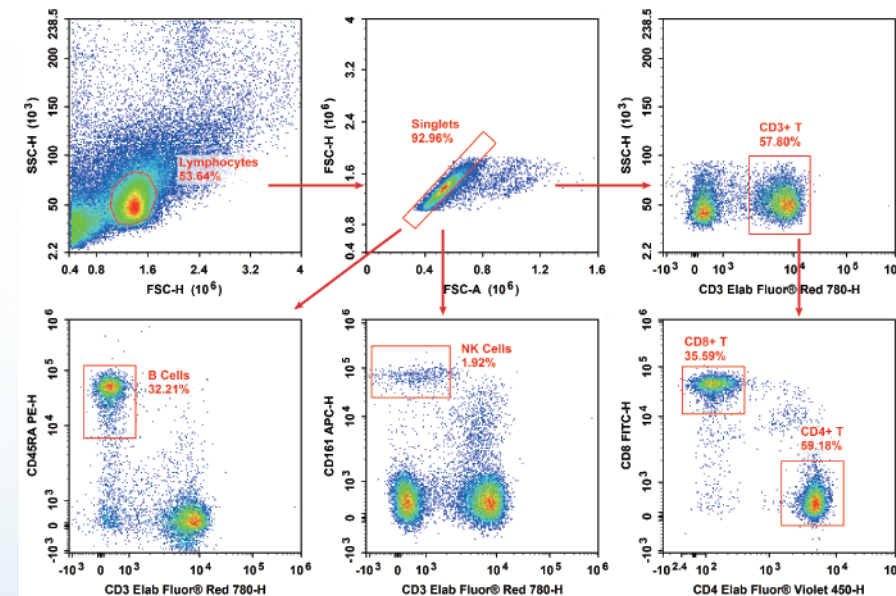
## Detection of T/B/NK (5-color) in Rat Spleen

### 01 Panel Design

Purpose	Sample	Antibody Collocation
Adjust the voltage	1	Blank
Adjust compensation	2	CD3-Elab Fluor® Red 780
	3	CD4-Elab Fluor® Violet 450
	4	CD8-FITC
	5	CD45RA-PE
	6	CD161-APC
APC-FMO in combination with Isotype Control for auxiliary gating	7	CD3-Elab Fluor® Red 780, CD4-Elab Fluor® Violet 450, CD8-FITC, CD45RA-PE, Mouse IgG1, κ Isotype Control-APC
PE-FMO in combination with Isotype Control for auxiliary gating	8	CD3-Elab Fluor® Red 780, CD4-Elab Fluor® Violet 450, CD8-FITC, CD161-APC; Mouse IgG1, κ Isotype Control-PE
Full Panel	9	CD3-Elab Fluor® Red 780, CD4-Elab Fluor® Violet 450, CD8-FITC, CD45RA-PE, CD161-APC

### 02 Information of Flow Cytometry Antibodies

Marker	Fluorochrome	Clone No.	Cat. No.
CD3	Elab Fluor® Red 780	G4.18	E-AB-F1228S
CD4	Elab Fluor® Violet 450	OX-38	E-AB-F1105Q
CD8	FITC	OX-8	E-AB-F1098C
CD45RA	PE	OX-33	E-AB-F1306D
CD161	APC	3.2.3	E-AB-F1307E
Mouse IgG1, κ Isotype Control	PE	MOPC-21	E-AB-F09792D
Mouse IgG1, κ Isotype Control	APC	MOPC-21	E-AB-F09792E



### Tips:

- ⊞ The CD3/CD4/CD8 cells are easy to be distinguished, and even without an Isotype Control, they can effectively distinguish between positive and negative cell populations.
- ⊞ Isotype Control for CD45RA and CD161 is suggested for auxiliary gating.
- ⊞ The phenotype of rat B cells is CD3<sup>-</sup>CD45RA<sup>+</sup>, and the phenotype of NK cells is CD3<sup>-</sup>CD161<sup>+</sup>.



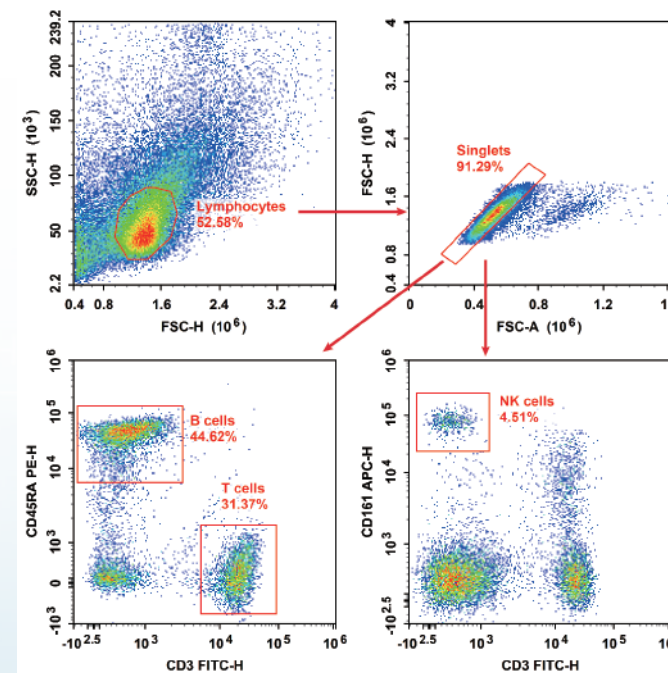
## Detection of T/B/NK (3-color) in Rat Spleen

### 01 Panel Design

Purpose	Sample	Antibody Collocation
Adjust the voltage	1	Blank
Adjust compensation	2	CD3-FITC
	3	CD45RA-PE
	4	CD161-APC
APC-FMO in combination with Isotype Control for auxiliary gating	5	CD3-FITC, CD45RA-PE; Mouse IgG1, κ Isotype Control-APC
PE-FMO in combination with Isotype Control for auxiliary gating	6	CD3-FITC, CD161-APC; Mouse IgG1, κ Isotype Control-PE
Full Panel	7	CD3-FITC, CD45RA-PE, CD161-APC

### 02 Information of Flow Cytometry Antibodies

Marker	Fluorochrome	Clone No.	Cat. No.
CD3	FITC	G4.18	E-AB-F1228C
CD45RA	PE	OX-33	E-AB-F1306D
CD161	APC	3.2.3	E-AB-F1307E
Mouse IgG1, κ Isotype Control	PE	MOPC-21	E-AB-F09792D
Mouse IgG1, κ Isotype Control	APC	MOPC-21	E-AB-F09792E



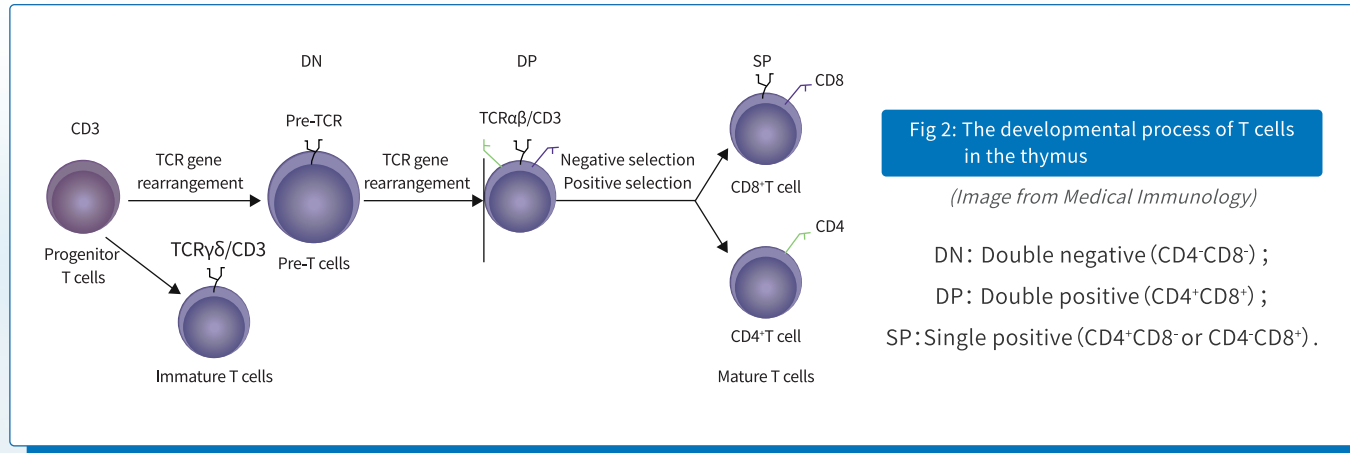
#### Tips:

- ⊞ The phenotype of rat B cells is CD3<sup>+</sup>CD45RA<sup>+</sup>, and the phenotype of NK cells is CD3<sup>+</sup>CD161<sup>+</sup>.
- ⊞ Isotype Control for CD45RA and CD161 is suggested for auxiliary gating.

## CD4<sup>+</sup>/CD8<sup>+</sup> T Cell Population Detection

According to function, T cells can be divided into the following categories:

- **CD4<sup>+</sup>T cell:** CD4<sup>+</sup> T cells secreting various cytokines to participate in immune function, is an important defense line for the body's antiviral and anti-tumor immunity.
- **CD8<sup>+</sup>T cell:** CD8<sup>+</sup> T cells secrete perforin, granzyme and other substances to directly kill cells, and induce apoptosis of target cells through Fas FasL pathway or TNF TNFR pathway.



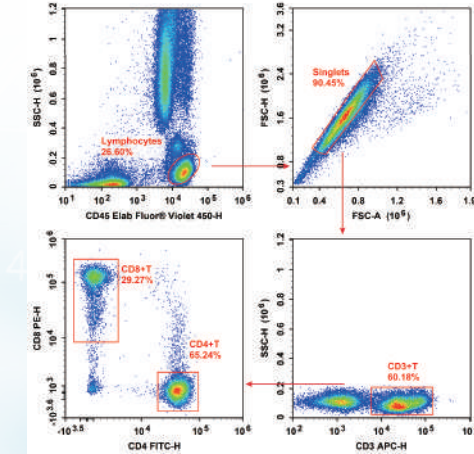
## Detection of T Cells (4-color) in Human Peripheral Blood

### 01 Panel Design

Purpose	Sample	Antibody Collocation
Adjust the voltage	1	Blank
Full Panel	2	CD45-Elab Fluor® Violet 450, CD3-APC, CD4-FITC, CD8a-PE

### 02 Information of Flow Cytometry Antibodies

Marker	Fluorochrome	Clone No.	Cat. No.
CD45	Elab Fluor® Violet 450	HI30	E-AB-F1137Q
CD3	APC	OKT3	E-AB-F1001E
CD4	FITC	RPA-T4	E-AB-F1109C
CD8a	PE	OKT-8	E-AB-F1110D



### Tips:

- ⊞ For human peripheral blood T cells, it is suggested to use CD45, which can easily gate the lymphocyte population.
- ⊞ The above panel shows clear cell populations, and there is no need to set single staining tubes for compensation.



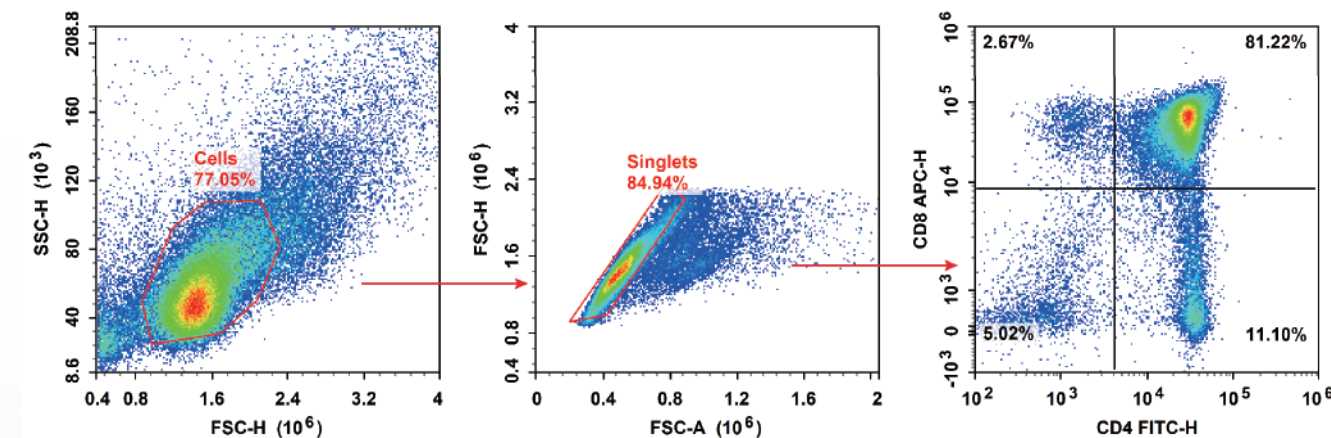
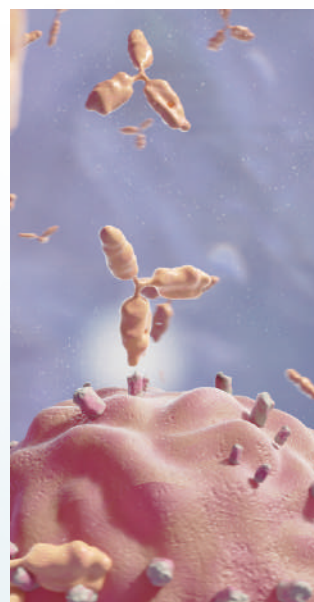
## 01 Detection of T Cells (2-color) in Mouse Thymus

### 01 Panel Design

Purpose	Sample	Antibody Collocation
Adjust the voltage	1	Blank
APC-FMO in combination with Isotype Control for auxiliary gating	2	CD4-FITC; Rat IgG2a, $\kappa$ Isotype Control-APC
FITC-FMO in combination with Isotype Control for auxiliary gating	3	CD8a-APC; Rat IgG2b, $\kappa$ Isotype Control- FITC
Full Panel	4	CD4-FITC, CD8a-APC

### 02 Information of Flow Cytometry Antibodies

Marker	Fluorochrome	Clone No.	Cat. No.
CD4	FITC	GK1.5	E-AB-F1097C
CD8a	APC	53-6.7	E-AB-F1104E
Rat IgG2b, $\kappa$ Isotype Control	FITC	LTF-2	E-AB-F09842C
Rat IgG2a, $\kappa$ Isotype Control	APC	2A3	E-AB-F09832E



#### Tips:

- According to the expression of CD4 and CD8, T cells in the thymus can be classified into double negative cells (DN cells: CD4<sup>-</sup>CD8<sup>-</sup>), double positive cells (DP cells: CD4<sup>+</sup>CD8<sup>+</sup>) and single positive cell (SP cell: CD4<sup>+</sup>CD8<sup>-</sup> or CD4<sup>-</sup>CD8<sup>+</sup>).
- The majority of T cells in the thymus co-express CD4 and CD8. Choosing two non-interference fluorescent combinations, FITC and APC, during panel design can reduce the complexity of data analysis.
- There is no interference between FITC and APC, so there is no need to set a single positive control compensation for this experiment.

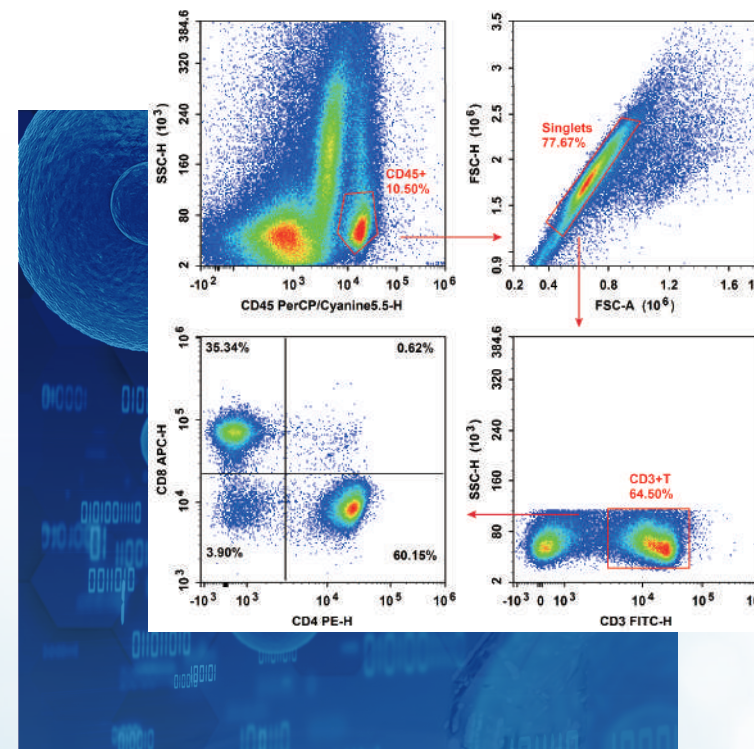
## ■ Detection of Lymphocyte (4 colors) in Mouse Tumor

### 01 Panel Design

Purpose	Sample	Antibody Collocation
Adjust the voltage	1	Blank
Adjust compensation	2	CD45-PerCP/Cyanine5.5
	3	CD3-FITC
	4	CD4-PE
	5	CD8-APC
Full Panel	6	CD45-PerCP/Cyanine5.5, CD3-FITC, CD4-PE, CD8a-APC

### 02 Information of Flow Cytometry Antibodies

Marker	Fluorochrome	Clone No.	Cat. No.
CD45	PerCP/Cyanine5.5	30-F11	E-AB-F1136J
CD3	FITC	17A2	E-AB-F1013C
CD4	PE	GK1.5	E-AB-F1097D
CD8a	APC	53-6.7	E-AB-F1104E



### Tips:

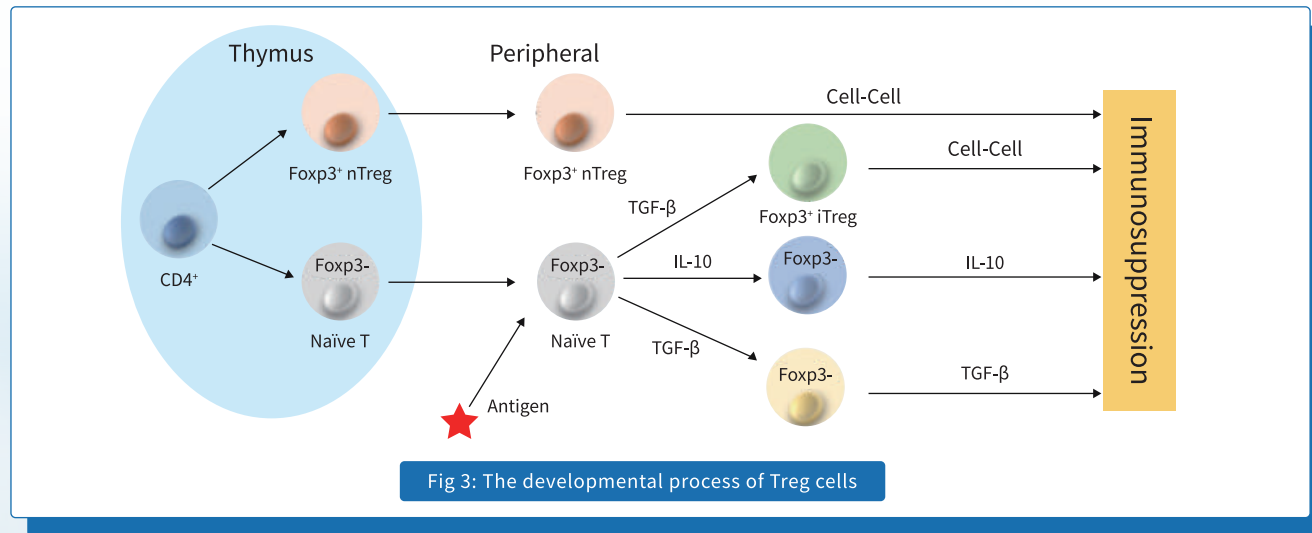
- ⊞ In tumor tissue, the majority cells are tumor cells, and the proportion of lymphocytes is relatively low. CD45 and SSC can be used to gate the lymphocytes.
- ⊞ The lymphocyte gate in tumor cells is defined as the gate of CD45<sup>high</sup> and SSC<sup>low</sup> on the CD45/SSC scatter plot.





## Treg Cell Population Detection

Treg (regulatory T cells) is a regulatory factor of the immune system, and it is crucial for maintaining self-tolerance and immune cell homeostasis. Treg plays an important role in immune tolerance, autoimmune diseases, infectious diseases, organ transplantation, tumors and other diseases. At present, the most commonly used markers for identifying Tregs are CD4, CD25, CD127, and Foxp3.



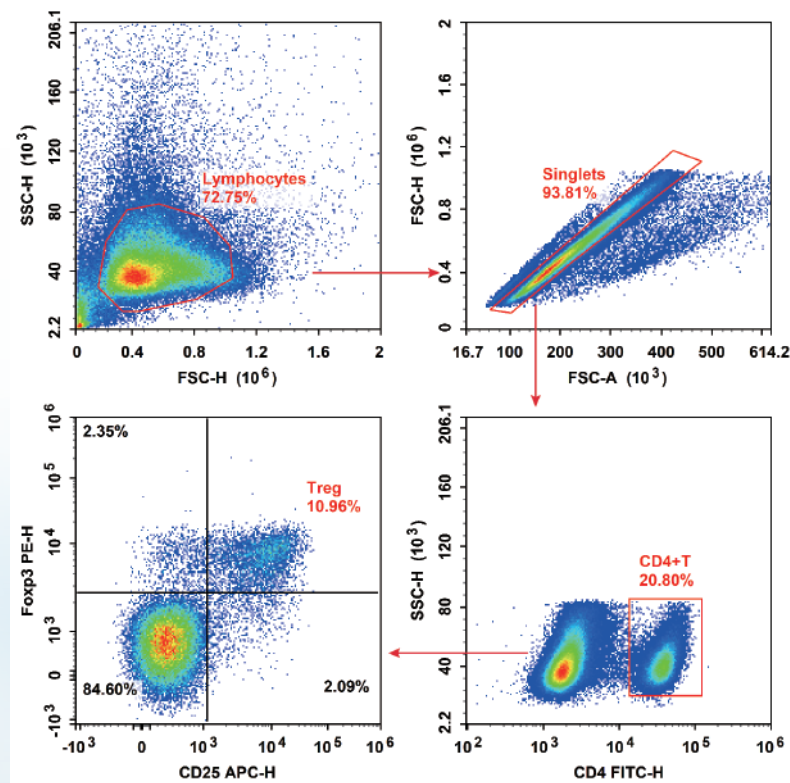
## Detection of Treg (3-color) in Mouse Spleen

### 01 Panel Design

Purpose	Sample	Antibody Collocation
Adjust the voltage	1	Blank
Adjust compensation	2	CD4-FITC
	3	CD25-APC
	4	Foxp3-PE
APC-FMO in combination with Isotype Control for auxiliary gating	5	CD4-FITC, Foxp3-PE; Rat IgG1, κ Isotype Control-APC
PE-FMO in combination with Isotype Control for auxiliary gating	6	CD4-FITC, CD25-APC; Mouse IgG1, κ Isotype Control-PE
Full Panel	7	CD4-FITC, CD25-APC, Foxp3-PE

### 02 Information of Flow Cytometry Antibodies

Marker	Fluorochrome	Clone No.	Cat. No.
CD4	FITC	GK1.5	E-AB-F1097C
CD25	APC	PC-61.5.3	E-AB-F1102E
Foxp3	PE	3G3	E-AB-F1238D
Rat IgG1, κ Isotype Control	APC	HRPN	E-AB-F09822E
Mouse IgG1, κ Isotype Control	PE	MOPC-21	E-AB-F09792D



**Tips:**

- ⊞ Mouse Treg marker is CD4<sup>+</sup> CD25<sup>+</sup> Fxp3<sup>+</sup>.
- ⊞ CD4 cell population is obvious, and there is no need of Isotype Control. But CD25 and Fxp3 populations are not obvious, and Isotype Controls are needed.
- ⊞ There is fluorescence spillover, so it is necessary to set single positive tubes for compensation.
- ⊞ Please be careful that inappropriate use of Fixation/Permeabilization buffer may cause high background and unclear cell clustering.

## Detection of Treg (6-color) in Human Peripheral Blood

### 01 Panel Design

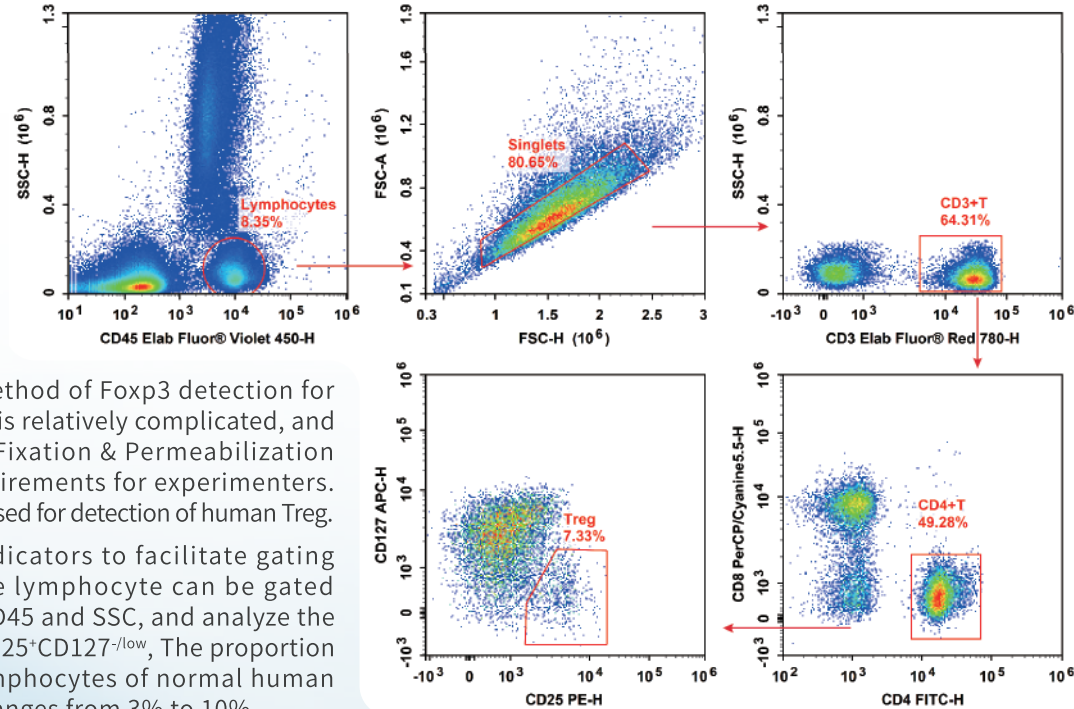
Purpose	Sample	Antibody Collocation
Adjust the voltage	1	Blank
Adjust compensation	2	CD45-Elab Fluor® Violet 450
	3	CD3-Elab Fluor® Red 780
	4	CD4-FITC
	5	CD8a-PerCP/Cyanine5.5
	6	CD25-PE
	7	CD127-Elab Fluor®647
	Full Panel	8

### 02 Information of Flow Cytometry Antibodies

Marker	Fluorochrome	Clone No.	Cat. No.
CD45	Elab Fluor® Violet 450	HI30	E-AB-F1137Q
CD3	Elab Fluor® Red 780	OKT3	E-AB-F1001S
CD4	FITC	RPA-T4	E-AB-F1109C
CD8a	PerCP/Cyanine5.5	OKT-8	E-AB-F1110J
CD25	PE	BC96	E-AB-F1194D
CD127	Elab Fluor®647	A019D5	E-AB-F1152M

**Tips:**

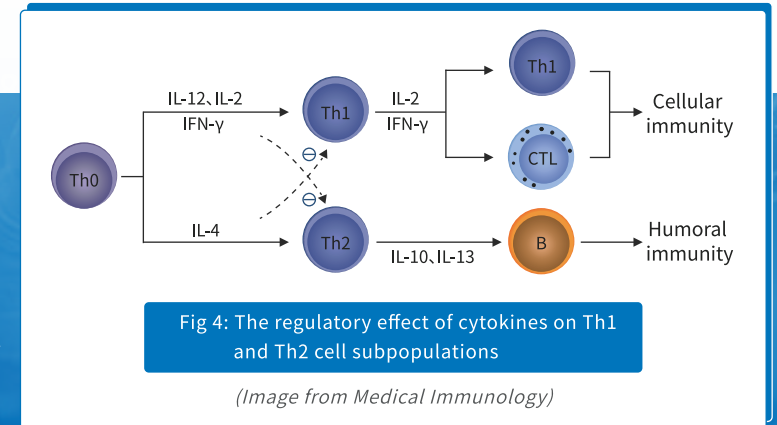
- ⊞ The traditional method of Foxp3 detection for Treg identification is relatively complicated, and the procedure of Fixation & Permeabilization requires high requirements for experimenters. Currently, CD127 is used for detection of human Treg.
- ⊞ Increase CD45 indicators to facilitate gating lymphocytes. The lymphocyte can be gated directly through CD45 and SSC, and analyze the cell ratio of CD4<sup>+</sup>CD25<sup>+</sup>CD127<sup>-/low</sup>, The proportion of Treg cells in lymphocytes of normal human peripheral blood ranges from 3% to 10%.
- ⊞ This scheme needs to set single positive tubes for compensation adjustment.



## Th1/Th2/ Th17 Cell Population Detection

Th cells (helper T cells) mainly activate macrophages and other immune cells to phagocytose and clear antigens through different subpopulations and interactions. Its main classifications include Th1, Th2, Th17, etc.

- **Th1 cell:** Cytokines secreted by Th1 enhance cell-mediated anti-infective immunity.
- **Th2 cell:** Th2 assists in the activation of B cells, and its secreted cytokines can also promote B cell proliferation, differentiation, and antibody generation. Th2 also plays an important role in hypersensitivity and anti-parasitic infections.





- **Th17 cell:** Th17 cells can secrete various cytokines involved in innate immunity and the occurrence of certain inflammations. Research has shown that Th17 cells have different functions in both pathogenic and non-pathogenic aspects, playing an important regulatory role in autoimmunity.

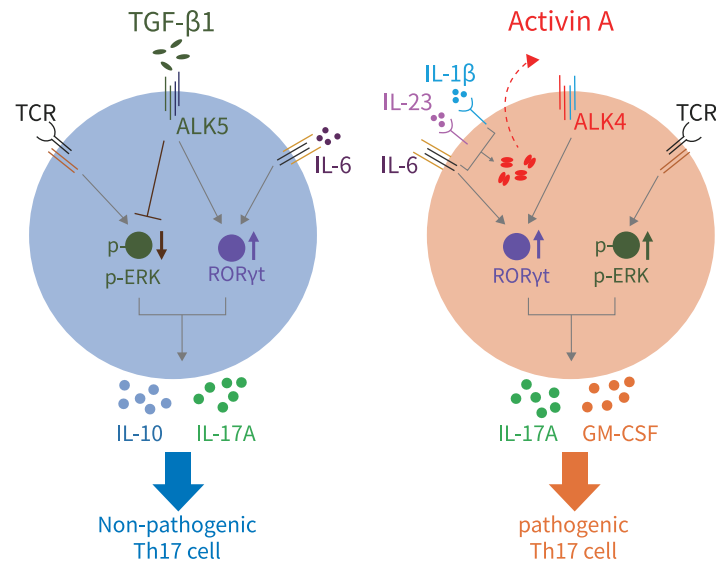


Fig 5: Differentiation and function of Th17 cells

(Picture source:Wu B,Zhang S,Guo Z,et al.The TGF-beta superfamily cytokine Activin-A is induced during autoimmune neuroinflammation and drives pathogenic Th17 cell differentiation [J]. *Immunity* 2021(2):54.)

## Detection of Th1/Th2 (4-color) in Human PBMC

### 01 Panel Design

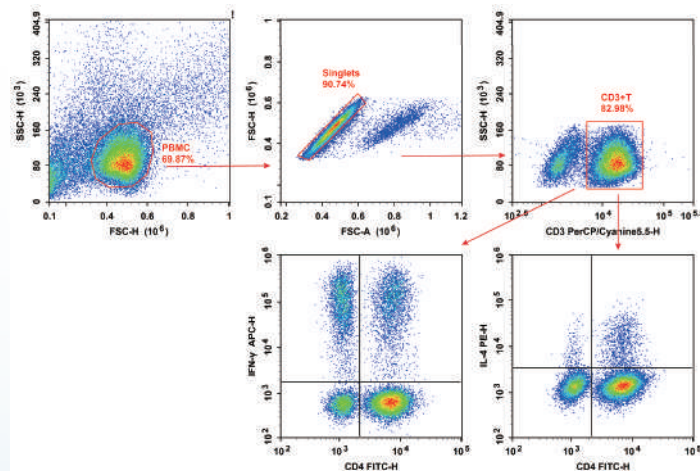
Purpose	Sample	Antibody Collocation
Adjust the voltage	1	Blank
Adjust compensation	2	CD3-PerCP/Cyanine5.5
	3	CD4-FITC
	4	IFN-γ-APC
	5	IL-4-PE
APC-FMO in combination with Isotype Control for auxiliary gating	6	CD3-PerCP/Cyanine5.5, CD4-FITC, IL-4-PE; Mouse IgG1, κ Isotype Control-APC
PE-FMO in combination with Isotype Control for auxiliary gating	7	CD3-PerCP/Cyanine5.5, CD4-FITC, IFN-γ-APC; Rat IgG1, κ Isotype Control-PE
Full Panel	8	CD3-PerCP/Cyanine5.5, CD4-FITC, IFN-γ-APC, IL-4-PE

### 02 Information of Flow Cytometry Antibodies

Marker	Fluorochrome	Clone No.	Cat. No.
CD3	PerCP/Cyanine5.5	UCHT1	E-AB-F1230J
CD4	FITC	SK3	E-AB-F1352C
IFN-γ	APC	B27	E-AB-F1196E
IL-4	PE	MP4-25D2	E-AB-F1203D
Mouse IgG1, κ Isotype Control	APC	MOPC-21	E-AB-F09792E
Rat IgG1, κ Isotype Control	PE	HRPN	E-AB-F09822D

**Tips:**

- After PBMC sorting, it is necessary to first use cytokine stimulating and blocking agents for stimulating and blocking culture (The reagents used in this experiment are: Cytokine Activation and Protein Blocking Kit (E-CK-A091). The cultivation conditions are as follows: after 1 hour of stimulation with a stimulating agent, add a blocking agent and incubate for another 4.5 hours), then collect cells for subsequent Flow Cytometry experiments.
- PMA stimulation can cause partial endocytosis of CD4 on the surface of human T cells, so we need to choose the CD4 clone SK3 with minimal impact on endocytosis.
- Isotype Controls for IFN- $\gamma$  and IL-4 are necessary, since the expression of cytokines is generally not high.
- CD3<sup>+</sup> CD4<sup>+</sup> IFN- $\gamma$ <sup>+</sup> is Th1 type, CD3<sup>+</sup> CD4<sup>+</sup> IL-4<sup>+</sup> is Th2 type.
- The Permeabilization buffer may cause significant damage to cells, so it is recommended that the cell precipitates formed after centrifugation should be dispersed into cell suspensions before adding the Permeabilization buffer to reduce cell damage.



## Detection of Th17 (3-color) in Human PBMC

### 01 Panel Design

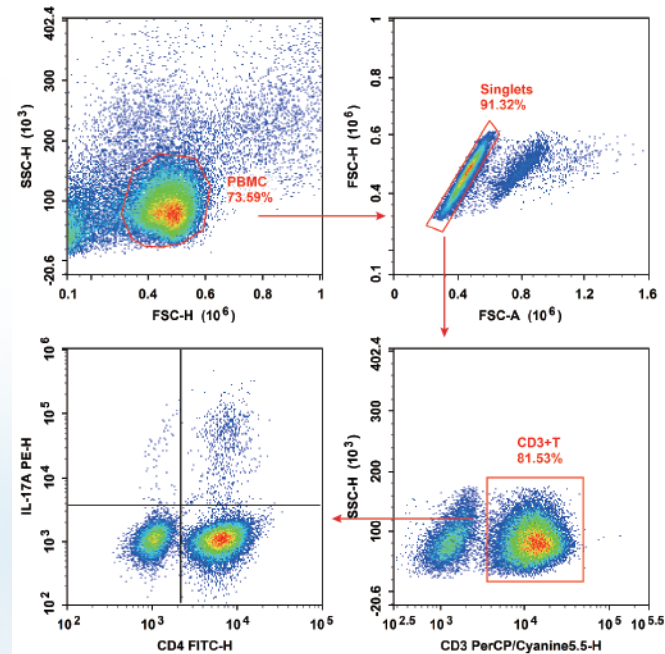
Purpose	Sample	Antibody Collocation
Adjust the voltage	1	Blank
Adjust compensation	2	CD3-PerCP/Cyanine5.5
	3	CD4-FITC
	4	IL-17A-PE
PE-FMO in combination with Isotype Control for auxiliary gating	5	CD3-PerCP/Cyanine5.5, CD4-FITC; Rat IgG1, $\kappa$ Isotype Control-PE
Full Panel	6	CD3-PerCP/Cyanine5.5, CD4-FITC, IL-17A-PE

### 02 Information of Flow Cytometry Antibodies

Marker	Fluorochrome	Clone No.	Cat. No.
CD3	PerCP/Cyanine5.5	UCHT1	E-AB-F1230J
CD4	FITC	SK3	E-AB-F1352C
IL-17A	PE	BL168	E-AB-F1173D
Mouse IgG1, $\kappa$ Isotype Control	PE	MOPC-21	E-AB-F09792D

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### Tips:



- ⊞ After PBMC sorting, it is necessary to first use cytokine stimulating and blocking agents for stimulating and blocking culture (The reagents used in this experiment are: Cytokine Activation and Protein Blocking Kit (E-CK-A091). The cultivation conditions are as follows: after 1 hour of stimulation with a stimulating agent, add a blocking agent and incubate for another 4.5 hours), then collect cells for subsequent Flow Cytometry experiments.
- ⊞ PMA stimulation can cause partial endocytosis of CD4 on the surface of human T cells, so we need to choose the CD4 clone SK3 with minimal impact on endocytosis.
- ⊞ Isotype control for IL-17A is necessary, since the expression of cytokines is generally not high.
- ⊞ CD3<sup>+</sup>CD4<sup>+</sup> IL-17A<sup>+</sup> is Th17 type.
- ⊞ The Permeabilization buffer may cause significant damage to cells, so it is recommended that the cell precipitates formed after centrifugation should be dispersed into cell suspensions before adding the Permeabilization buffer to reduce cell damage.

## Flow Cytometry Staining Protocol

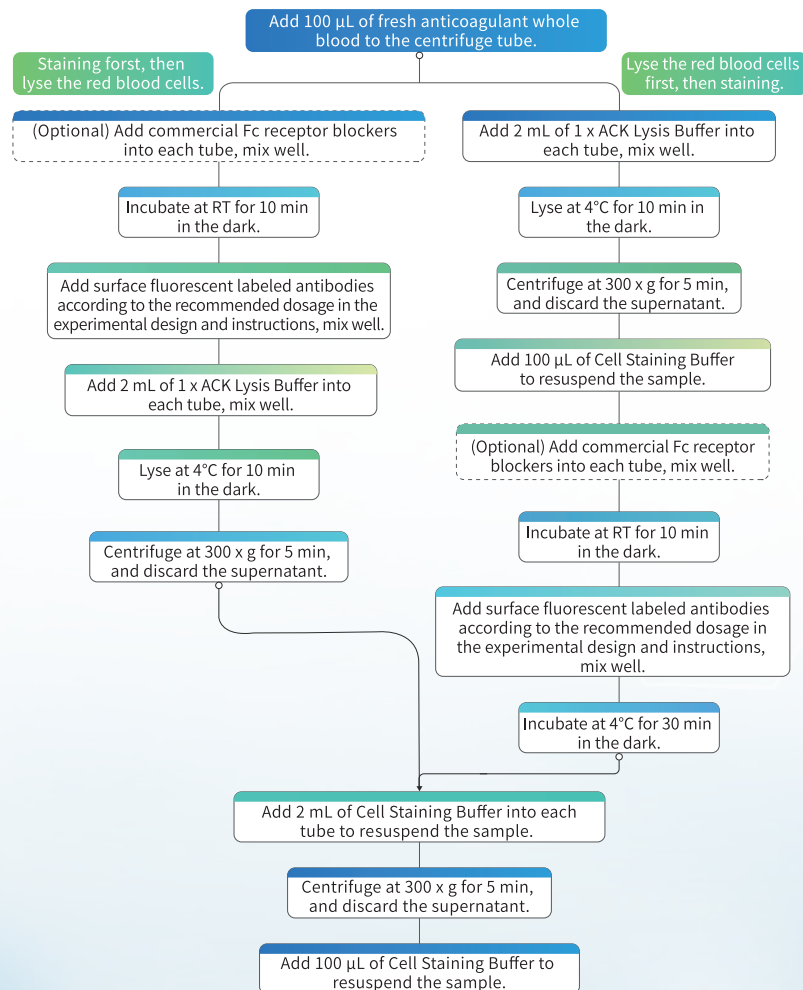
- ⊞ Cell Surface Targets Staining for Flow Cytometry
  - Human whole blood staining process
  - Single cell suspension staining process
- ⊞ Cells Intracellular Targets Staining for Flow Cytometry
- ⊞ Cells Intranuclear Targets Staining for Flow Cytometry

The staining process of Flow Cytometry may vary depending on the expression site of the detection indicators. Elabscience® provides the corresponding Flow Cytometry staining protocols according to the expression position of the indicators for reference by customers.



## Cell Surface Targets Staining for Flow Cytometry

### Human whole blood staining process



### Single cell suspension staining process

1. Prepare the required tissues for the experiment (spleen, bone marrow, lymph nodes, thymus, etc.) into a single-cell suspension.
2. After counting the suspension with a hemocytometer or other instruments, adjust the cell concentration to about  $1 \times 10^7/\text{mL}$ .
3. Add 100  $\mu\text{L}$  of cell suspension (approximately  $1 \times 10^6$  cells) to each tube according to the experiment design.
4. (Blocking Fc receptor, optional) Blocking Fc receptors may reduce nonspecific during the staining process.

**For mouse samples**, purified Anti-Mouse CD16/CD32 antibody specific for Fc $\gamma$ R III/II can be used to block nonspecific staining of antibodies, and reduces the background fluorescence of negative cells to the level of unlabeled cells. 1  $\mu\text{g}$  of purified anti-mouse CD16/32 monoclonal antibody (E-AB-F0997A) was added and incubated at room temperature for 10 min.

**For rat samples**, excessive purified Ig from the same source and subtype as fluorescent antibodies or serum from the same source can be directly used for blocking, or commercial FcR blocking agents can be used.

**For human samples**, purified CD16 mab can be used as an FcR blocking agent. 1  $\mu\text{g}$  of purified anti-human CD16 monoclonal antibody (E-AB-F1236A) was added and incubated at room temperature for 10 min.

5. Add surface fluorescent labeled antibodies according to the recommended dosage in the experimental design and instructions, mix well, and incubate at 4°C for 30 min in the dark.
6. Add at least 2 mL of Cell Staining Buffer to resuspend cells. Centrifuge at 300 x g for 5 min, and discard the supernatant.
7. Add 200  $\mu\text{L}$  of Cell Staining Buffer to resuspend the sample, detect by Flow Cytometer.

## Cells Intracellular Targets Staining for Flow Cytometry

1. Prepare the experimental sample (spleen, bone marrow, lymph nodes, thymus, etc.) into a single-cell suspension.

\*\* If the target indicator is cytokine, the cells should be stimulated and blocked to prevent the transport of secreted proteins to the extracellular.

### ● Protocol of stimulating and blocking: (It is recommended to use E-CK-A091)

- 1) Prepare the single cell suspension with complete medium (self-prepared), and adjust the cell density to  $1\sim 2 \times 10^6/\text{mL}$ .
  - 2) Add 2  $\mu\text{L}$  of 500 x Cell Stimulation MIX to each 1 mL of cell suspension, and incubate the cells at  $37^\circ\text{C}$ ,  $5\%\text{CO}_2$  for 4~18 h.
  - 3) Add 1  $\mu\text{L}$  of 1000 x Protein Transport Inhibitor MIX to each 1mL of cell suspension, and incubate the cells at  $37^\circ\text{C}$ ,  $5\%\text{CO}_2$  for 5~16 h.
  - 4) Collect cell suspension, centrifuge at  $200\sim 300 \times g$  for 5 min, and discard the supernatant.
  - 5) Resuspend the sample with Cell Staining Buffer.
2. Count the suspension with a hemocytometer or other instruments, adjust the cell concentration to about  $1 \times 10^7/\text{mL}$ .
  3. Add 100  $\mu\text{L}$  of cell suspension (approximately  $1 \times 10^6$  cells) to each tube.
  4. (Optional) Perform a Fixed Viability Dye according to the instructions.

5. (Blocking Fc receptor, optional) Blocking Fc receptors may reduce nonspecific during the staining process.

**For mouse samples**, purified Anti-Mouse CD16/CD32 antibody specific for Fc $\gamma$ R III/II can be used to block nonspecific staining of antibodies, and reduces the background fluorescence of negative cells to the level of unlabeled cells. 1  $\mu\text{g}$  of purified anti-mouse CD16/32 monoclonal antibody (E-AB-F0997A) was added and incubated at room temperature for 10 min.

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6. Add surface fluorescent labeled antibodies according to the recommended dosage in the experimental design and instructions, mix well, and incubate at  $4^\circ\text{C}$  for 30 min in the dark.
7. Add 2 mL of Cell Staining Buffer to resuspend cells. Centrifuge at  $300 \times g$  for 5 min, and discard the supernatant.
8. Add 200  $\mu\text{L}$  of Cell Staining Buffer to resuspend the sample. Add 200  $\mu\text{L}$  of 1 x Fixation Buffer to each tube, mix gently. Incubate at RT for 30~60 min in the dark.
9. Add 1 mL of 1 x Permeabilization Working Solution into each tube, mix gently. Centrifuge at  $600 \times g$  for 5 min, and discard the supernatant.
10. Add 100  $\mu\text{L}$  of 1 x Permeabilization Working Solution into each tube to resuspend the sample. And add corresponding intracellular detection antibody according to the recommended dosage in the experimental design and instructions. Incubate at RT for 30 min in the dark.
11. Add 2 mL of Cell Staining Buffer. Centrifuge at  $600 \times g$  for 5 min and discard the supernatant.
12. Add 200  $\mu\text{L}$  of Cell Staining Buffer to resuspend the sample, detect by Flow Cytometer.

## Cells Intranuclear Targets Staining for Flow Cytometry

1. Prepare the experimental sample (spleen, bone marrow, lymph nodes, thymus, etc.) into a single-cell suspension.
2. Count the suspension with a hemocytometer or other instruments, adjust the cell concentration to about  $1 \times 10^7/\text{mL}$ .
3. Add 100  $\mu\text{L}$  of cell suspension (approximately  $1 \times 10^6$  cells) to each tube.
4. (Optional) Perform a Fixed Viability Dye according to the instructions.
5. (Blocking Fc receptor, optional) Blocking Fc receptors may reduce nonspecific during the staining process.

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6. Add surface fluorescent labeled antibodies according to the recommended dosage in the experimental design and instructions, mix well, and incubate at 4°C for 30 min in the dark.

7. Add 1 mL of Cell Staining Buffer to each tube, centrifuge at 300 x g for 5 min, and discard the supernatant. Then add 100  $\mu\text{L}$  of Cell Staining Buffer to resuspend the cells.

8. Prepare 1 x Fixation and Permeabilization Buffer (It is recommended to use **E-CK-A108**):

**1 x Fixation Working Solution:** Dilute Fixation Concentrate (4x) with Fixation Dilution Solution to 1x Fixation Working Solution.

**1 x Permeabilization Working Solution:** Dilute Permeabilization Buffer (10x) with ddH<sub>2</sub>O to 1x Permeabilization Working Solution.

9. Add 1 mL of 1 x Fixation Working Solution to each tube and mix well, incubate the cells at 4°C for 30 min, then centrifuge at 600 x g for 5 min and discard the supernatant.
10. Add 2 mL of 1 x Permeabilization Working Solution to each tube and mix well, centrifuge at 600 x g for 5 min and discard the supernatant.
11. Repeat Step 10.
12. Resuspend the cells with 100  $\mu\text{L}$  of 1 x Permeabilization Working Solution. And add corresponding antibody according to the recommended dosage in the experimental design and instructions. Incubate at RT for 30 min in the dark.
13. Add 2 mL of 1 x Permeabilization Working Solution to each tube and centrifuge at 600 x g for 5 min at RT. Discard the supernatant.
14. Add 200  $\mu\text{L}$  of Cell Staining Buffer to resuspend the sample, detect by Flow Cytometer.

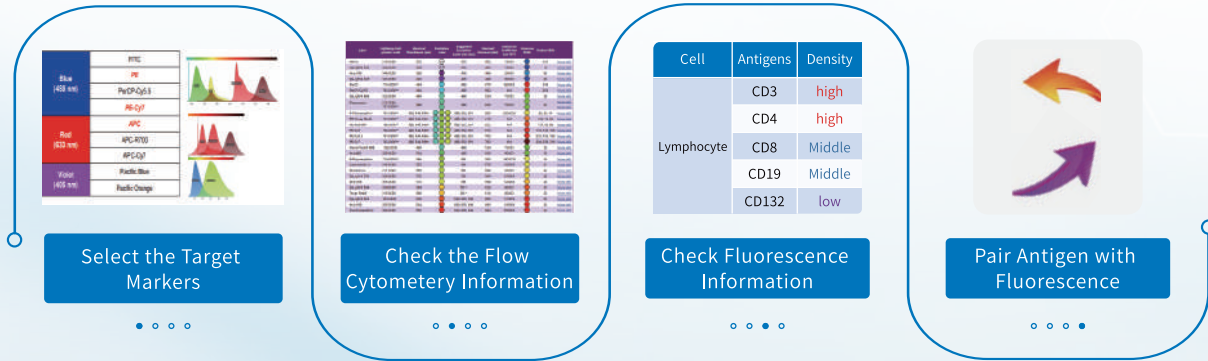


# Elabscience® Featured Services

## Elabscience® Panel Design

Elabscience® provides customers with professional and free Panel Design Services. You only need to provide Flow Cytometry experimental indicators (logical relationships or references, expression levels of target marker) and basic information of Flow Cytometer (laser, detection channel, filter information), Elabscience® will provide professional and free Panel Design Services based on your experiments.

### Process of Panel Design Services



## Elabscience® Data Analysis

Customers using Elabscience® product can also provide FCS format data and indicator logical relationships to our technical support, Elabscience® can provide you with professional and free Data Analysis Services.

### Process of Data Analysis Services

