



## Elabscience Bionovation Inc.

Toll-free: 1-888-852-8623

Tel: 1-832-243-6086

Fax: 1-832-243-6017

Web: [www.elabscience.com](http://www.elabscience.com)

Email: [orders@elabscience.com](mailto:orders@elabscience.com); [techsupport@elabscience.com](mailto:techsupport@elabscience.com)

EBR085-2601v1

Elabscience®

# Phenotyping of Immune Cells by Flow Cytometer

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

🌐 A Reliable Research Partner in Life Science and Medicine

Elabscience Bionovation Inc.

# About Elabscience®

Elabscience® stands at the forefront of biotechnology innovation, expertly combining independent design, R&D, manufacturing, and sales to deliver premier reagents and services for cell detection research. Our diverse product portfolio includes advanced solutions for detecting membrane and intracellular proteins (Flow cytometry antibodies), secreted proteins (ELISA kits), cell glycolipid metabolic intermediates and inorganic salts (Metabolism Assays), and comprehensive assessments of cellular function and health (Cell Apoptosis Assay, Cell Cycle Assay, Cell Proliferation/Cytotoxicity/Viability).



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Technical  
Platforms

Note: The citations data is up to the end of December 2025.

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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

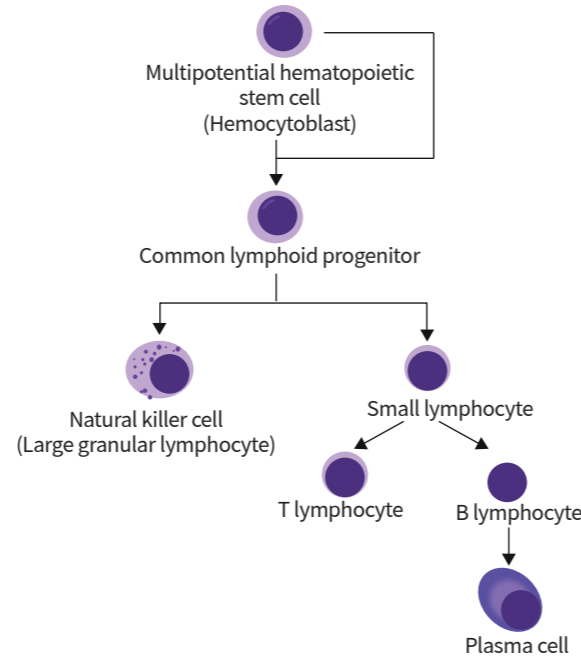


Fig. 1. Differentiation of lymphocytes

(Image source: *Medical Immunology*)

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

### 01 Panel Design

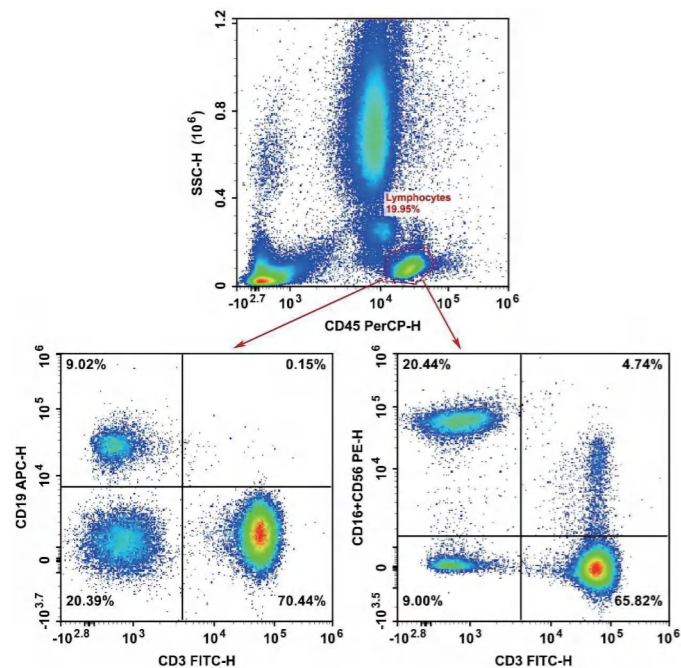
| Purpose   | Sample | Antibody Collocation   |
|---|--------|--|
| Adjust the voltage  | 1      | Blank  |
| Adjust compensation   | 2      | CD45-PerCP   |
|   | 3      | CD3-FITC   |
|   | 4      | CD16-PE, CD56-PE   |
| PE-FMO in combination with Isotype Control for auxiliary gating | 5      | CD19-APC   |
|   | 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:

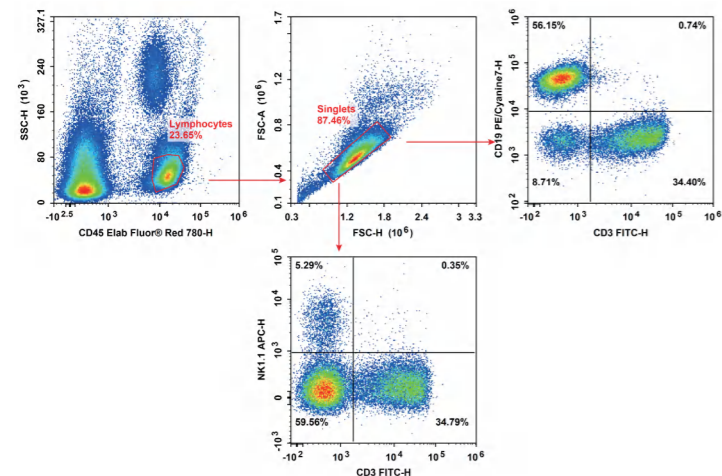
- ⊞ This panel has obvious cell populations, and compensation regulation can be performed without single positive tube. But for beginners of Flow Cytometry, it is recommended to set up a single positive tube to adjust compensation.
- ⊞ The detection standard for NK cells is CD3-CD16<sup>+</sup>CD56<sup>+</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  |
| Adjust compensation  | 2      | CD45-Elab Fluor® Red 780   |
|  | 3      | CD3-FITC   |
|  | 4      | CD19-PE/Cyanine7   |
| APC-FMO in combination with Isotype Control for auxiliary gating | 5      | NK1.1-APC  |
|  | 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<sup>+</sup>NK1.1<sup>+</sup>/CD3<sup>+</sup>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.

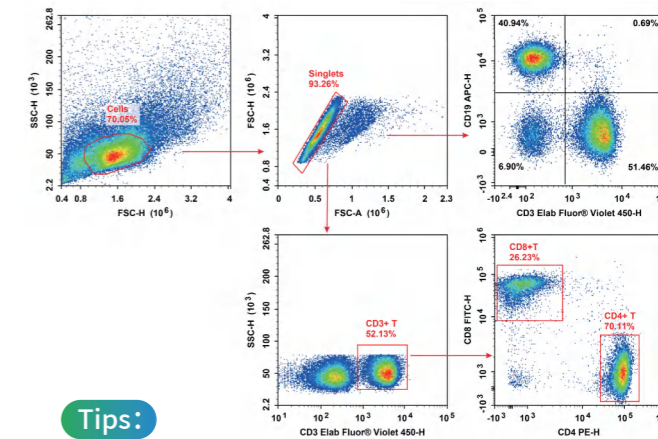
## Detection of T/B cells (4-color) in Mouse Lymph Node

### 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.
- ⊞ The CD3/CD4/CD8/CD19 cell populations are clearly distinct. While it is not necessary to use single-color stained tubes for compensation adjustment, FMO controls, or Isotype Controls for gating, it is highly recommended that beginners in Flow Cytometry set up single-color stained tubes for compensation adjustment.

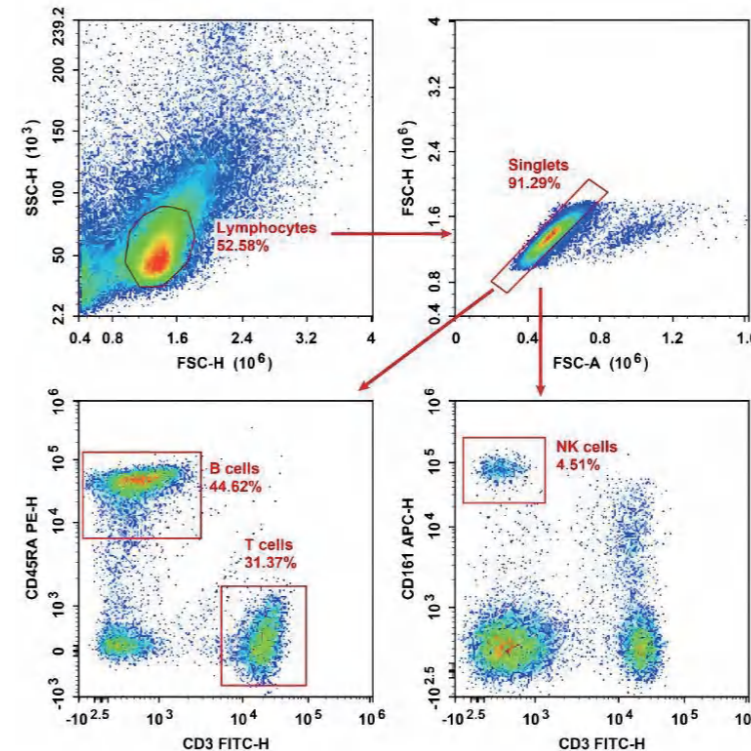
## Detection of T/B/NK (3-color) in Rat Spleen

### 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.

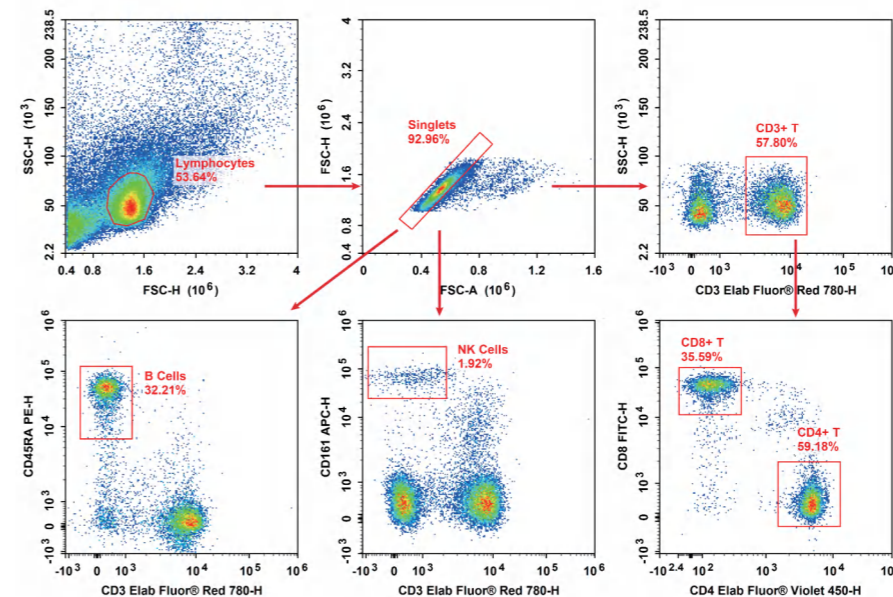
## Detection of T/B/NK (5-color) in Rat Spleen

### 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-CD45RA<sup>+</sup>, and the phenotype of NK cells is CD3-CD161<sup>+</sup>.

## 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.

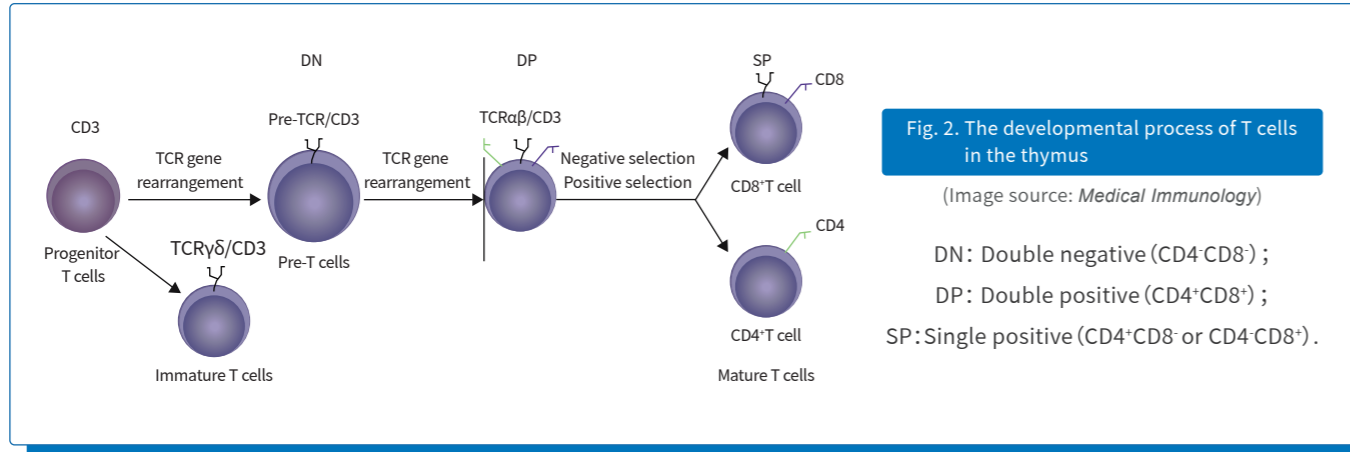


Fig. 2. The developmental process of T cells in the thymus

(Image source: Medical Immunology)

DN: Double negative (CD4<sup>-</sup>CD8<sup>-</sup>);  
 DP: Double positive (CD4<sup>+</sup>CD8<sup>+</sup>);  
 SP: Single positive (CD4<sup>+</sup>CD8<sup>-</sup> or CD4<sup>-</sup>CD8<sup>+</sup>).

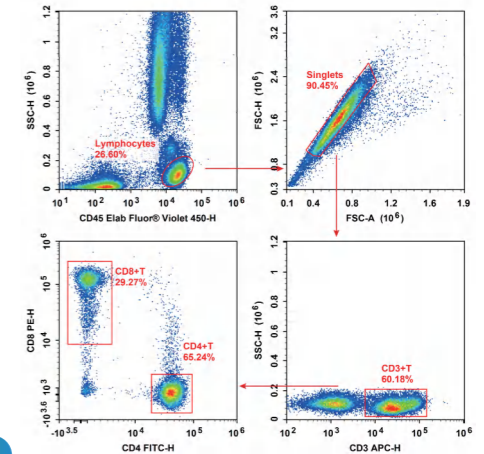
## Detection of T Cells (4-color) in Human Peripheral Blood

### 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.
- ⊞ For this panel design, cell populations are well-separated and single-stained tubes are not required for compensation. However, beginners in flow cytometry are strongly advised to include them.

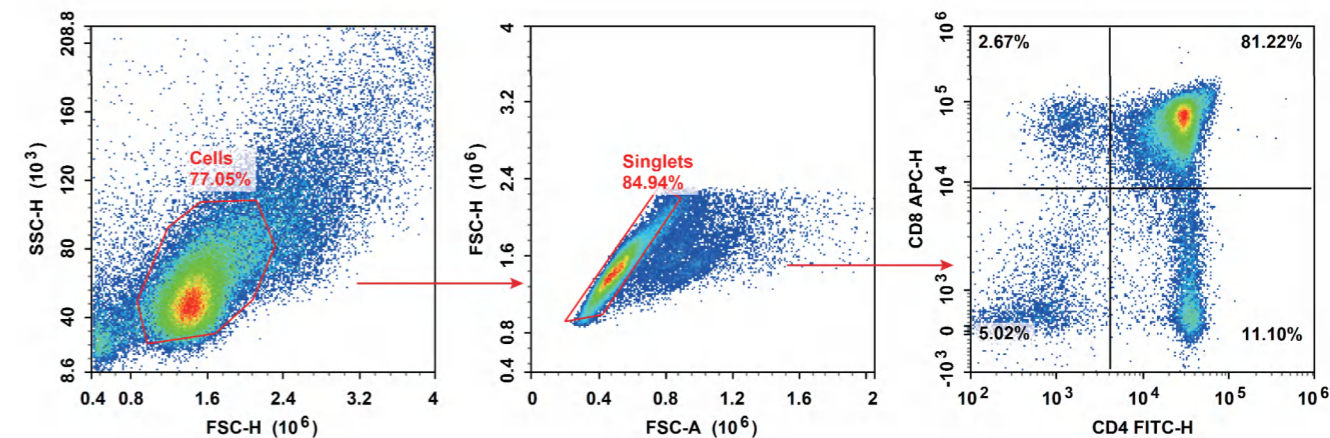
## 01 Detection of T Cells (2-color) in Mouse Thymus

### 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.

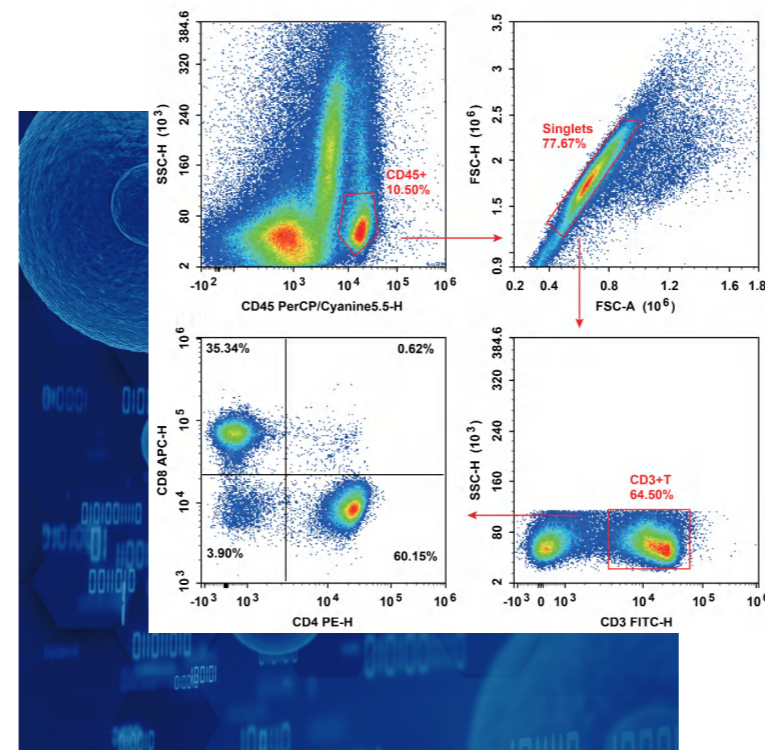
## 1 | Detection of Lymphocyte (4 colors) in Mouse Tumor

### 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      | CD8a-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.
- ⊞ This experimental protocol is applicable to the detection of lymphocytes in non-lymphoid or hematopoietic cancer samples.

## 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. At present, the most commonly used markers for identifying Tregs are CD4, CD25, CD127, and Foxp3. Treg plays an important role in immune tolerance, autoimmune diseases, infectious diseases, organ transplantation, tumors and other diseases.

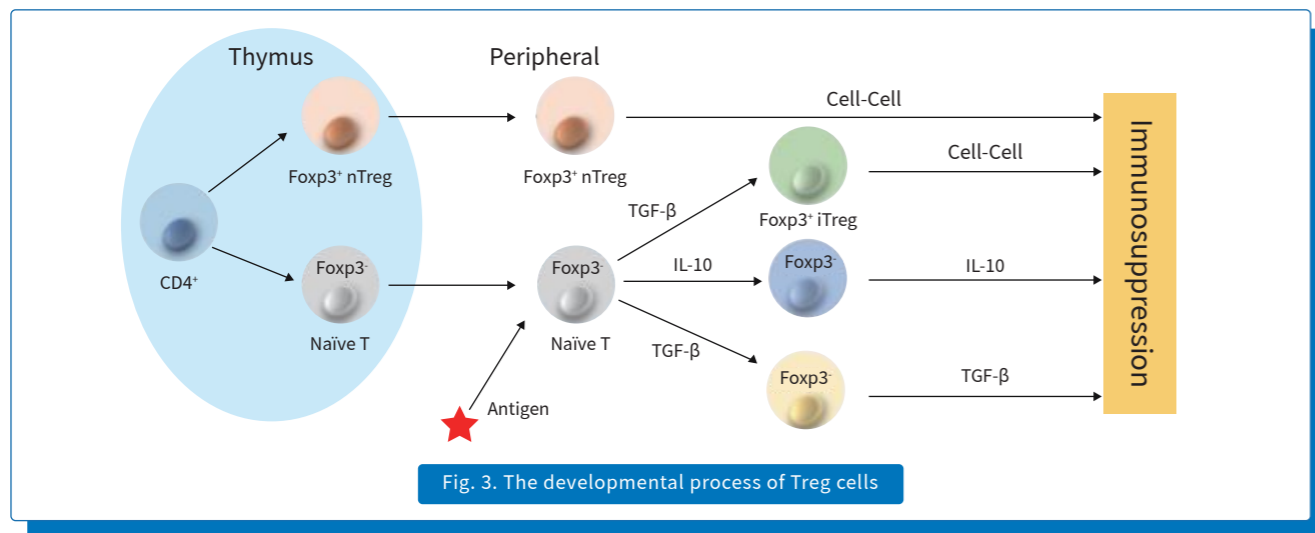


Fig. 3. The developmental process of Treg cells

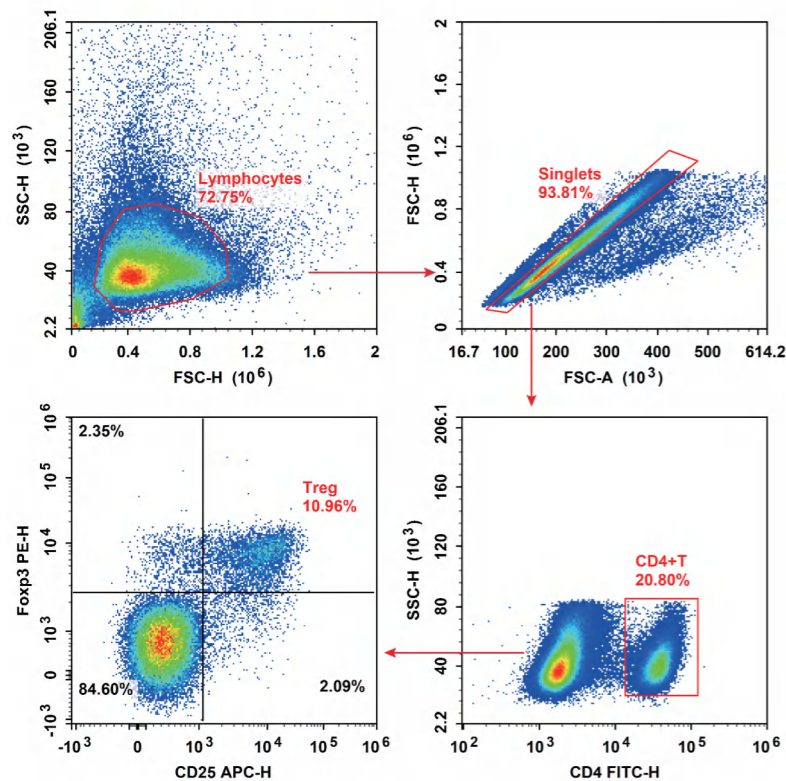
## Detection of Treg (3-color) in Mouse Spleen

### 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>Foxp3<sup>+</sup>.
- ⊞ CD4 cell population is obvious, and there is no need of Isotype Control. But CD25 and Foxp3 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

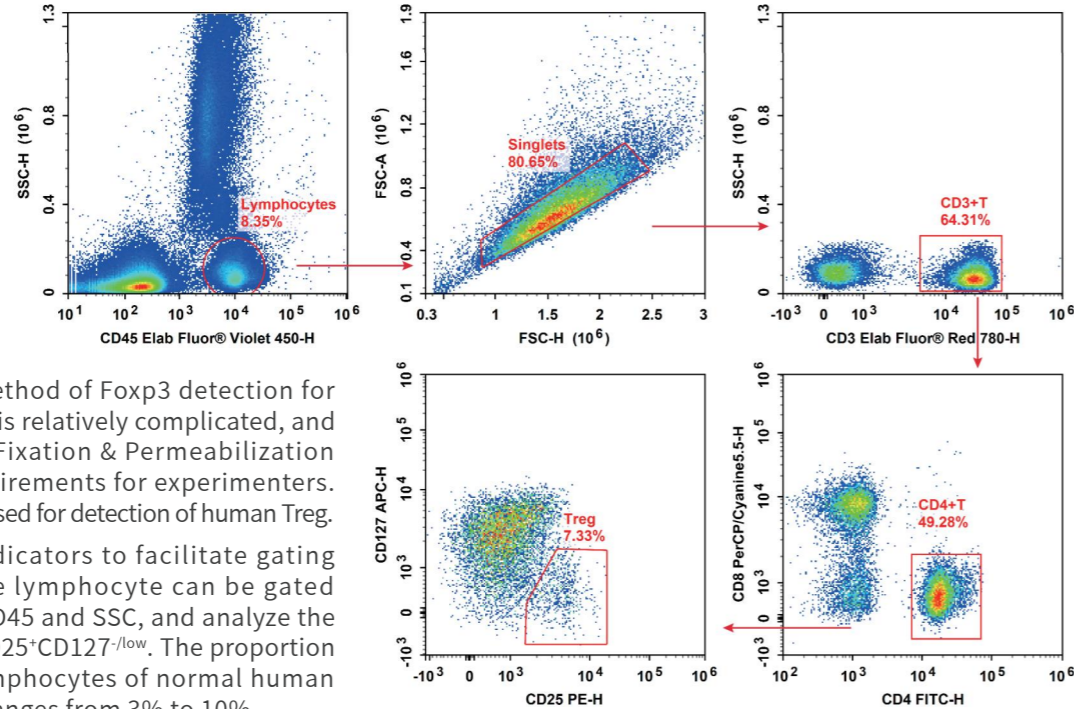
| Purpose             | Sample | Antibody Collocation   |
|---------------------|--------|--|
| Adjust the voltage  | 1      | Blank  |
| Adjust compensation | 2      | CD45-Elab Fluor® Violet 450  |
|                     | 3      | CD3-Elab Fluor® Red 780  |
|                     | 4      | CD4-FITC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

### 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:**

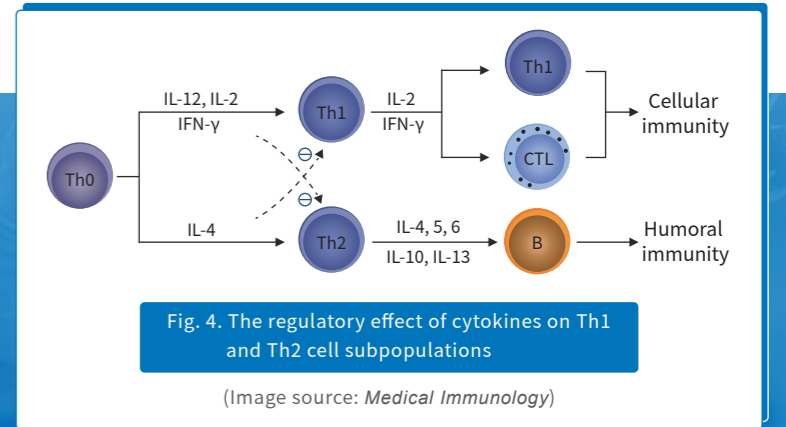
- ⊞ The traditional method of Foxp3 detection for Treg identification is relatively complicated, and the procedure of Fixation & Permeabilization requires high requirements for experimenters. Currently, CD127 is used for detection of human Treg.
- ⊞ Increase CD45 indicators to facilitate gating lymphocytes. 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.

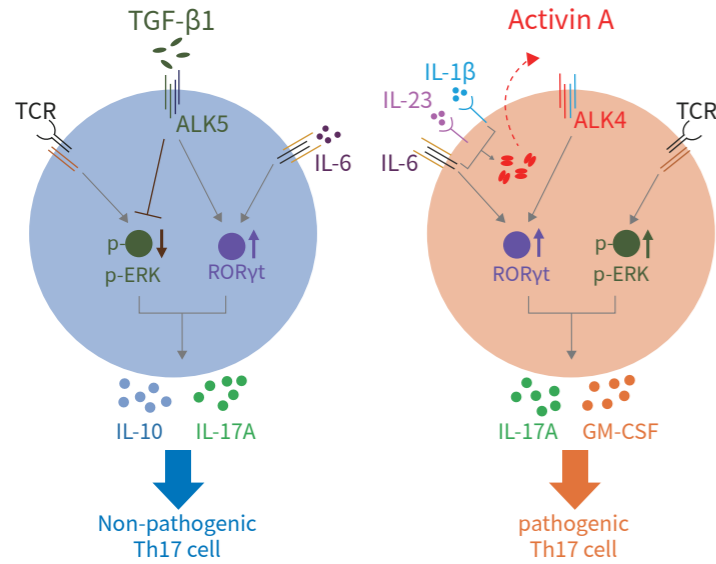


Fig. 5. Differentiation and function of Th17 cells

(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 Peripheral Blood PBMC

### 01 Panel Design

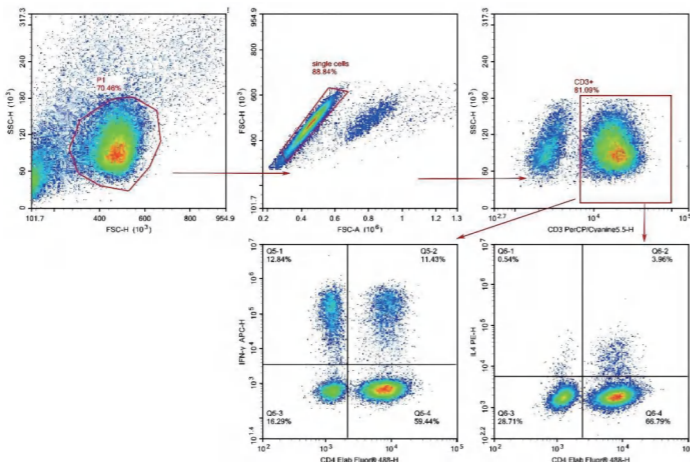
| Purpose  | Sample | Antibody Collocation  |
|--|--------|---|
| Adjust the voltage   | 1      | Blank   |
| Adjust compensation  | 2      | CD3-PerCP/Cyanine5.5  |
|  | 3      | CD4-Elab Fluor® 488   |
|  | 4      | IFN-γ-APC   |
|  | 5      | IL-4-PE   |
| APC-FMO in combination with Isotype Control for auxiliary gating | 6      | CD3-PerCP/Cyanine5.5, CD4-Elab Fluor® 488, 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-Elab Fluor® 488, IFN-γ-APC, Rat IgG1, κ Isotype Control-PE  |
| Full Panel   | 8      | CD3-PerCP/Cyanine5.5, CD4-Elab Fluor® 488, 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                           | Elab Fluor® 488  | SK3       | E-AB-F1352L  |
| 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 (for stimulation-blocking experimental conditions, please refer to the instructions of the selected kit).
- ⊞ 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-γ and IL-4 are necessary, since the expression of cytokines is generally not high.
- ⊞ CD3<sup>+</sup>CD4<sup>+</sup>IFN-γ<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.



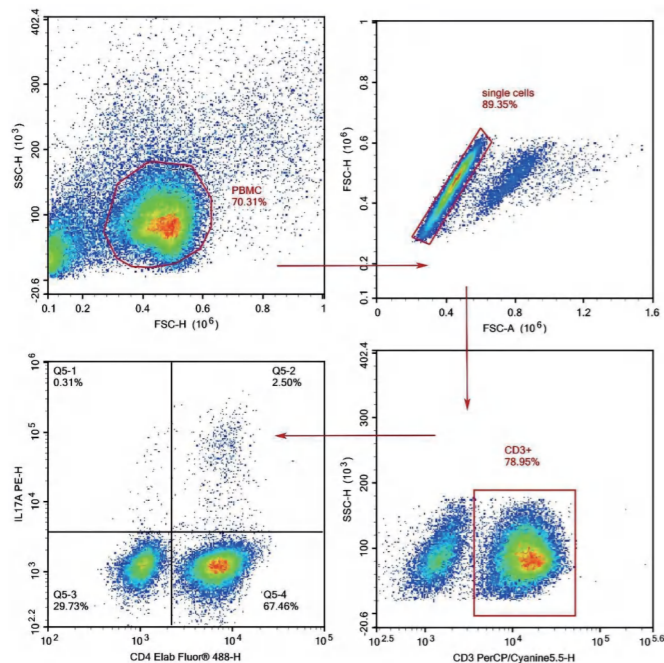
## Detection of Th17 (3-color) in Human Peripheral Blood PBMC

### 01 Panel Design

| Purpose   | Sample | Antibody Collocation  |
|---|--------|---|
| Adjust the voltage  | 1      | Blank   |
| Adjust compensation   | 2      | CD3-PerCP/Cyanine5.5  |
|   | 3      | CD4-Elab Fluor® 488   |
| PE-FMO in combination with Isotype Control for auxiliary gating | 4      | IL-17A-PE   |
|   | 5      | CD3-PerCP/Cyanine5.5, CD4-Elab Fluor® 488, Rat IgG1, κ Isotype Control-PE |
| Full Panel  | 6      | CD3-PerCP/Cyanine5.5, CD4-Elab Fluor® 488, IL-17A-PE                      |

### 02 Information of Flow Cytometry Antibodies

| Marker                        | Fluorochrome     | Clone No. | Cat. No.     |
|-------------------------------|------------------|-----------|--------------|
| CD3                           | PerCP/Cyanine5.5 | UCHT1     | E-AB-F1230J  |
| CD4                           | Elab Fluor® 488  | SK3       | E-AB-F1352L  |
| IL-17A                        | PE               | BL168     | E-AB-F1173D  |
| Mouse IgG1, κ Isotype Control | PE               | MOPC-21   | E-AB-F09792D |



**Tips:**

- ⊞ After PBMC sorting, it is necessary to first use cytokine stimulating and blocking agents for stimulating and blocking culture (for stimulation-blocking experimental conditions, please refer to the instructions of the selected kit).
- ⊞ 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.

**Detection of Th1/Th2 (4-color) in Mouse Spleen**

**01 Panel Design**

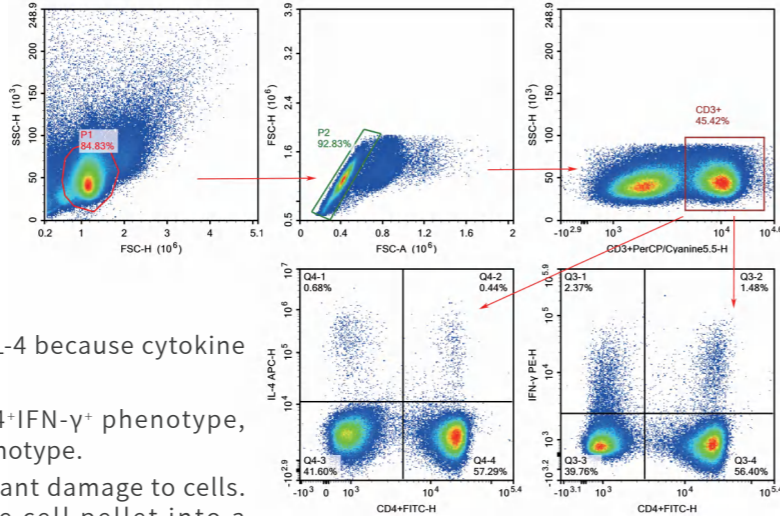
| Purpose  | Sample | Antibody Collocation  |
|--|--------|---|
| Adjust the voltage   | 1      | Blank   |
| Adjust compensation  | 2      | CD3-PerCP/Cyanine5.5  |
|  | 3      | CD4-FITC  |
|  | 4      | IFN-γ-PE  |
|  | 5      | IL-4-APC  |
| APC-FMO in combination with Isotype Control for auxiliary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

**02 Information of Flow Cytometry Antibodies**

| Marker                      | Fluorochrome     | Clone No. | Cat. No.     |
|-----------------------------|------------------|-----------|--------------|
| CD3                         | PerCP/Cyanine5.5 | 17A2      | E-AB-F1013J  |
| CD4                         | FITC             | RM4-5     | E-AB-F1353C  |
| IFN-γ                       | PE               | XMG1.2    | E-AB-F1101D  |
| IL-4                        | APC              | 11B11     | E-AB-F1204E  |
| Rat IgG1, κ Isotype Control | PE               | HRPN      | E-AB-F09822D |
| Rat IgG1, κ Isotype Control | APC              | HRPN      | E-AB-F09822E |

**Tips:**

- ⊞ After the preparation and counting of the splenic single-cell suspension are completed, it is necessary to perform stimulation and inhibition culture using cytokine stimulation inhibitors (for experimental conditions of stimulation and inhibition, please refer to the instructions of the reagent kit used).
- ⊞ PMA stimulation can induce partial internalization of CD4 on the surface of human T cells. Therefore, it is necessary to select the CD4 clone RM4-5, which has minimal impact on internalization.
- ⊞ Isotype controls are required for IFN-γ and IL-4 because cytokine expression levels are generally low.
- ⊞ Th1 cells are characterized by the CD3<sup>+</sup>CD4<sup>+</sup>IFN-γ<sup>+</sup> phenotype, while Th2 cells exhibit the CD3<sup>+</sup>CD4<sup>+</sup>IL-4<sup>+</sup> phenotype.
- ⊞ Permeabilization reagents can cause significant damage to cells. It is recommended to first resuspend the cell pellet into a single-cell suspension after centrifugation before adding the permeabilization reagent, in order to minimize cell loss.



**Detection of Th17 (3-color) in Mouse Spleen**

**01 Panel Design**

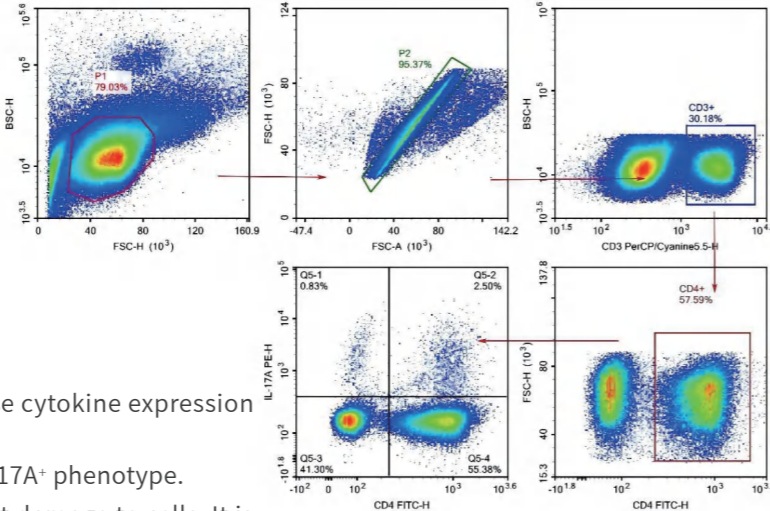
| Purpose   | Sample | Antibody Collocation   |
|---|--------|--|
| Adjust the voltage  | 1      | Blank  |
| Adjust compensation   | 2      | CD3-PerCP/Cyanine5.5   |
|   | 3      | CD4-FITC   |
|   | 4      | IL-17A-PE  |
| PE-FMO in combination with Isotype Control for auxiliary gating | 5      | CD3-PerCP/Cyanine5.5, CD4-FITC, Rat IgG1, κ 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 | 17A2         | E-AB-F1013J  |
| CD4                        | FITC             | RM4-5        | E-AB-F1353C  |
| IL-17A                     | PE               | TC11-18H10.1 | E-AB-F1199D  |
| Rat IgG1,κ Isotype Control | PE               | HRPN         | E-AB-F09822D |

**Tips:**

- ⊞ After the preparation and counting of the splenic single-cell suspension are completed, it is necessary to perform stimulation and inhibition culture using cytokine stimulation inhibitors (for experimental conditions of stimulation and inhibition, please refer to the instructions of the reagent kit used).
- ⊞ PMA stimulation can induce partial internalization of CD4 on the surface of human T cells. Therefore, it is necessary to select the CD4 clone RM4-5, which has minimal impact on internalization.
- ⊞ Isotype controls are required for IL-17A because cytokine expression levels are generally low.
- ⊞ Th17 cells are characterized by the CD3<sup>+</sup>CD4<sup>+</sup>IL-17A<sup>+</sup> phenotype.
- ⊞ Permeabilization reagents can cause significant damage to cells. It is recommended to first resuspend the cell pellet into a single-cell suspension after centrifugation before adding the permeabilization reagent, in order to minimize cell loss.



## DC Cell Population Detection

**Dendritic cells (DCs)**, named for their unique morphology, are the most potent antigen-presenting cells. They are widely distributed and currently recognized as the most powerful antigen-presenting cells. Moreover, they are the only antigen-presenting cells capable of activating naïve T cells, thereby initiating primary T cell immune responses. Recent studies have shown that DCs play a significant role in anti-tumor immunity, anti-infection responses, and autoimmune diseases. Research on DCs, particularly in the field of DC-based tumor vaccines, has become a major focus in biology and medicine.

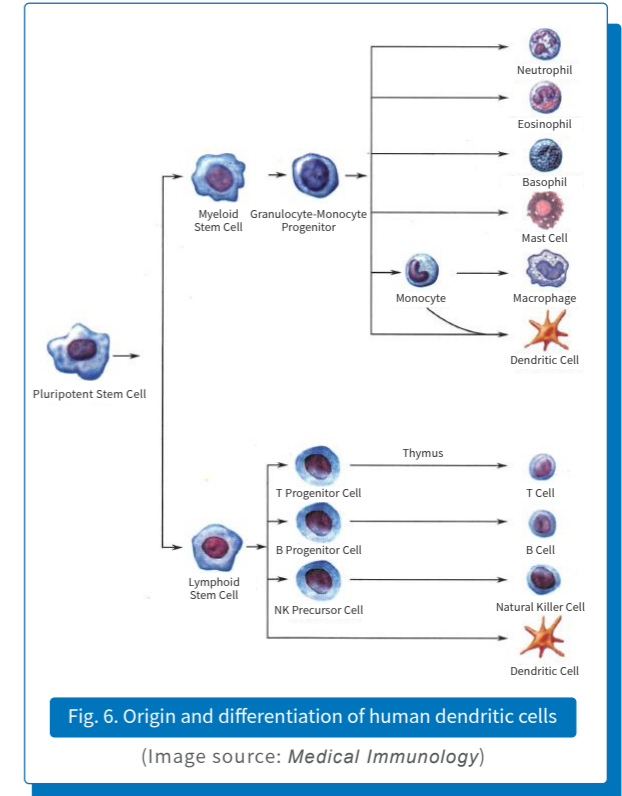


Fig. 6. Origin and differentiation of human dendritic cells

(Image source: *Medical Immunology*)

## 1 Detection of Dendritic Cell (5-Color) in Human Peripheral Blood

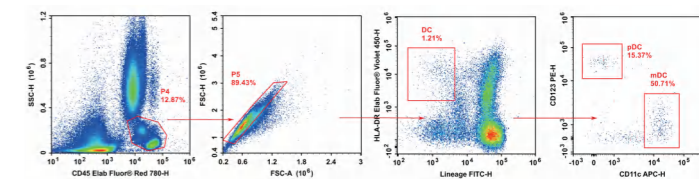
### 01 Panel Design

| Purpose   | Sample | Antibody Collocation  |
|---|--------|---|
| Adjust the voltage  | 1      | Blank   |
| Adjust compensation   | 2      | CD45-Elab Fluor® Red 780  |
|   | 3      | CD3-FITC, CD19-FITC, CD56-FITC, CD14-FITC   |
|   | 4      | HLA-DR-Elab Fluor® Violet 450   |
|   | 5      | CD123-PE  |
| FITC-FMO in combination with Isotype Control for auxiliary gating                   | 6      | CD11c-APC   |
|   | 7      | CD45-Elab Fluor® Red 780, HLA-DR-Elab Fluor® Violet 450, CD123-PE, CD11c-APC, Mouse IgG2a, κ Isotype Control-FITC, Mouse IgG1, κ Isotype Control-FITC |
| Elab Fluor® Violet 450-FMO in combination with Isotype Control for auxiliary gating | 8      | CD45-Elab Fluor® Red 780, CD3-FITC, CD19-FITC, CD56-FITC, CD14-FITC, CD123-PE, CD11c-APC, Mouse IgG2a, κ Isotype Control-Elab Fluor® Violet 450       |

| Purpose  | Sample | Antibody Collocation  |
|--|--------|---|
| PE-FMO in combination with Isotype Control for auxiliary gating  | 9      | CD45-Elab Fluor® Red 780, CD3-FITC, CD19-FITC, CD56-FITC, CD14-FITC, HLA-DR-Elab Fluor® Violet 450, CD11c-APC, Mouse IgG1, κ Isotype Control-PE |
| APC-FMO in combination with Isotype Control for auxiliary gating | 10     | CD45-Elab Fluor® Red 780, CD3-FITC, CD19-FITC, CD56-FITC, CD14-FITC, HLA-DR-Elab Fluor® Violet 450, CD123-PE, Mouse IgG1, κ Isotype Control-APC |
| Full Panel   | 11     | CD45-Elab Fluor® Red 780, CD3-FITC, CD19-FITC, CD56-FITC, CD14-FITC, HLA-DR-Elab Fluor® Violet 450, CD123-PE, CD11c-APC                         |

### 02 Information of Flow Cytometry Antibodies

| Marker                        | Fluorochrome           | Clone No. | Cat. No.     |
|-------------------------------|------------------------|-----------|--------------|
| CD45                          | Elab Fluor® Red 780    | HI30      | E-AB-F1137S  |
| CD3                           | FITC                   | OKT3      | E-AB-F1001C  |
| CD19                          | FITC                   | CD19      | E-AB-F1004C  |
| CD56                          | FITC                   | MY31      | E-AB-F1270C  |
| CD14                          | FITC                   | M5E2      | E-AB-F1209C  |
| HLA-DR                        | Elab Fluor® Violet 450 | L243      | E-AB-F1111Q  |
| CD123                         | PE                     | 6H6       | E-AB-F1117D  |
| CD11c                         | APC                    | BU15      | E-AB-F1118E  |
| Mouse IgG2a,κ Isotype Control | FITC                   | C1.18.4   | E-AB-F09802C |
| Mouse IgG1,κ Isotype Control  | FITC                   | MOPC-21   | E-AB-F09792C |
| Mouse IgG2a,κ Isotype Control | Elab Fluor® Violet 450 | C1.18.4   | E-AB-F09802Q |
| Mouse IgG1,κ Isotype Control  | PE                     | MOPC-21   | E-AB-F09792D |
| Mouse IgG1,κ Isotype Control  | APC                    | MOPC-21   | E-AB-F09792E |



#### Tips:

- ⊞ Dendritic cells (DCs) in human peripheral blood are classified into plasmacytoid dendritic cells (pDCs) and myeloid dendritic cells (mDCs).
- ⊞ pDC: lin(CD3, CD19, CD56, CD14)·HLA-DR<sup>+</sup>CD123<sup>+</sup>CD11c<sup>-</sup>;
- ⊞ mDC: lin(CD3, CD19, CD56, CD14)·HLA-DR<sup>+</sup>CD123<sup>+</sup>CD11c<sup>+</sup>.
- ⊞ Dendritic cells (DCs) account for a very low percentage in whole blood and are usually difficult to detect directly. Therefore, an alternative approach is to isolate peripheral blood mononuclear cells (PBMCs) first prior to detection.
- ⊞ The staining procedure for the FMO (Fluorescence Minus One) control, used in conjunction with the Isotype Control, is the same as that for the full panel sample, except that the staining for one target marker is omitted and replaced with the corresponding Isotype Control. This control reveals the background fluorescence contributed by all reagents except for that specific one, thereby providing more accurate guidance for setting the gating boundaries.

## Detection of Dendritic Cell (4-Color) in Mouse Spleen

### 01 Panel Design

| Purpose   | Sample | Antibody Collocation  |
|---|--------|---|
| Adjust the voltage  | 1      | Blank   |
| Adjust compensation   | 2      | CD3-FITC  |
|   | 3      | MHC II-Elab Fluor® Violet 450   |
|   | 4      | CD11c-PE  |
| Elab Fluor® Violet 450-FMO in combination with Isotype Control for auxiliary gating | 5      | CD11b-APC   |
|   | 6      | CD3-FITC, CD11c-PE, CD11b-APC, Rat IgG2b, κ Isotype Control-Elab Fluor® Violet 450          |
| PE-FMO in combination with Isotype Control for auxiliary gating                     | 7      | CD3-FITC, MHC II-Elab Fluor® Violet 450, CD11b-APC, Armenian Hamster IgG Isotype Control-PE |
| APC-FMO in combination with Isotype Control for auxiliary gating                    | 8      | CD3-FITC, MHC II-Elab Fluor® Violet 450, CD11c-PE, Rat IgG2b, κ Isotype Control-APC         |

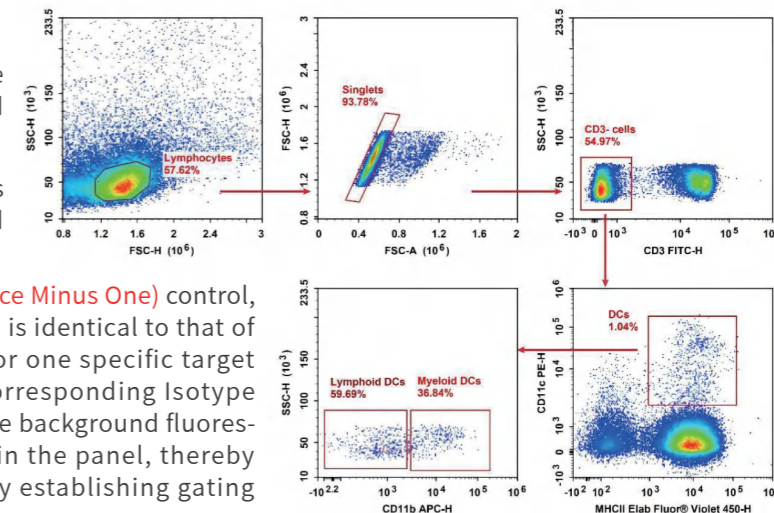
| Purpose    | Sample | Antibody Collocation   |
|------------|--------|--|
| Full Panel | 9      | CD3-FITC, MHC II-Elab Fluor® Violet 450, CD11c-PE, CD11b-APC |

### 02 Information of Flow Cytometry Antibodies

| Marker                               | Fluorochrome           | Clone No. | Cat. No.     |
|--------------------------------------|------------------------|-----------|--------------|
| CD3                                  | FITC                   | 17A2      | E-AB-F1013C  |
| MHC II                               | Elab Fluor® Violet 450 | M5/114    | E-AB-F0990Q  |
| CD11c                                | PE                     | N418      | E-AB-F0991D  |
| CD11b                                | APC                    | M1/70     | E-AB-F1081E  |
| Rat IgG2b,κ Isotype Control          | Elab Fluor® Violet 450 | LTF-2     | E-AB-F09842Q |
| Armenian Hamster IgG Isotype Control | PE                     | PIP       | E-AB-F09852D |
| Rat IgG2b,κ Isotype Control          | APC                    | LTF-2     | E-AB-F09842E |

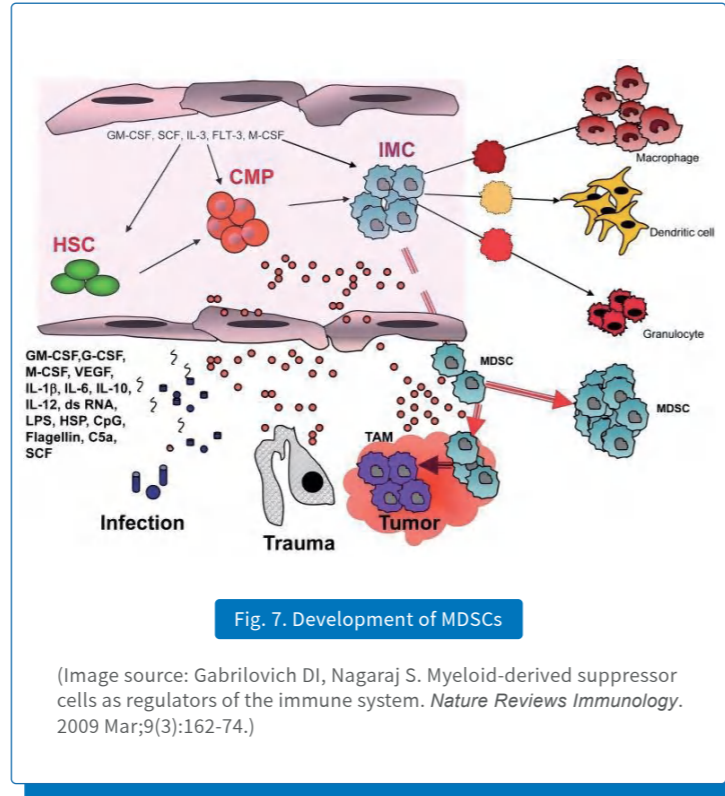
### Tips:

- ⊞ Dendritic cells (DCs) in the mouse spleen are classified into lymphoid dendritic cells and myeloid dendritic cells.
- ⊞ The phenotype of lymphoid dendritic cells is CD11c<sup>+</sup>MHCII<sup>-</sup>CD11b<sup>-</sup>, while that of myeloid dendritic cells is CD11c<sup>+</sup>MHCII<sup>+</sup>CD11b<sup>+</sup>.
- ⊞ The staining protocol for the FMO (Fluorescence Minus One) control, used in conjunction with an Isotype Control, is identical to that of the test sample, except that the staining for one specific target marker is omitted and replaced with its corresponding Isotype Control. This FMO setup reveals the collective background fluorescence contributed by all other antibodies in the panel, thereby providing a critical reference for accurately establishing gating boundaries.



## MDSC Cell Population Detection

**MDSCs** (Myeloid-derived suppressor cells) are a group of suppressor cells derived from the bone marrow. They are precursors to dendritic cells (DCs), macrophages, and/or granulocytes, possessing a remarkable ability to suppress immune cell responses. By hindering the body's anti-tumor immunity, they play a critical role in promoting tumor immune escape and metastasis, making them one of the hotspots in current cancer research. In mice, MDSCs are classified into granulocytic MDSCs (G-MDSCs) and monocytic MDSCs (M-MDSCs). The following section introduces two detection methods.



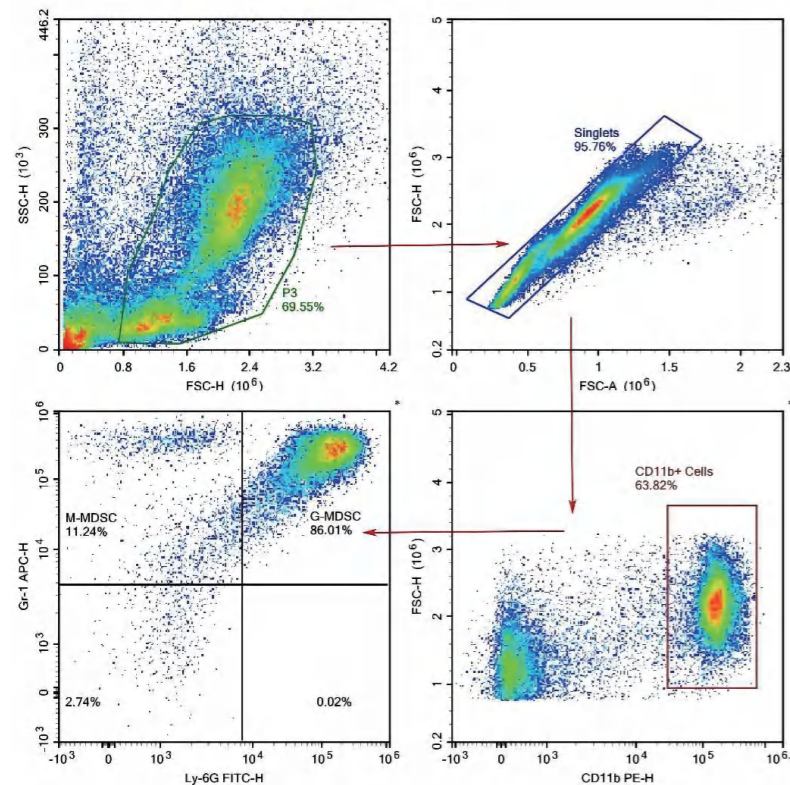
## Detection of MDSC (3-Color) in Mouse Bone Marrow (Panel 1)

### 01 Panel Design

| Purpose   | Sample | Antibody Collocation                                  |
|---|--------|---|
| Adjust the voltage  | 1      | Blank   |
|   | 2      | CD11b-PE  |
| Adjust compensation   | 3      | Ly-6G-FITC  |
|   | 4      | Gr-1-APC  |
| PE-FMO in combination with Isotype Control for auxiliary gating   | 5      | Ly-6G-FITC, Gr-1-APC, Rat IgG2b,k Isotype Control-PE  |
| FITC-FMO in combination with Isotype Control for auxiliary gating | 6      | CD11b-PE, Gr-1-APC, Rat IgG2a,k Isotype Control-FITC  |
| APC-FMO in combination with Isotype Control for auxiliary gating  | 7      | CD11b-PE, Ly-6G-FITC, Rat IgG2b,k Isotype Control-APC |
| Full Panel  | 8      | CD11b-PE, Ly-6G-FITC, Gr-1-APC                        |

### 02 Information of Flow Cytometry Antibodies

| Marker                      | Fluorochrome | Clone No. | Cat. No.     |
|-----------------------------|--------------|-----------|--------------|
| CD11b                       | PE           | M1/70     | E-AB-F1081D  |
| Ly-6G                       | FITC         | 1A8       | E-AB-F1108C  |
| Gr-1                        | APC          | RB6-8C5   | E-AB-F1120E  |
| Rat IgG2b,k Isotype Control | PE           | LTF-2     | E-AB-F09842D |
| Rat IgG2a,k Isotype Control | FITC         | 2A3       | E-AB-F09832C |
| Rat IgG2b,k Isotype Control | APC          | LTF-2     | E-AB-F09842E |



**Tips:**

- ⊞ Murine MDSCs are divided into granulocytic MDSCs (g-MDSCs, CD11b<sup>+</sup>Gr-1<sup>+</sup>Ly6G<sup>+</sup>) and monocytic MDSCs (m-MDSCs, CD11b<sup>+</sup>Gr-1<sup>-</sup>Ly6G<sup>-</sup>).
- ⊞ The Gr-1 antibody recognizes both Ly6G and Ly6C. In this experiment, MDSCs are classified based on the expression profiles of Gr-1 and Ly6G.
- ⊞ In this experiment, CD11b-positive cells were gated to analyze the expression of Gr-1 and Ly6G. The Gr-1<sup>+</sup>Ly6G<sup>-</sup> population was identified as monocytic MDSCs (M-MDSCs), while the Gr-1<sup>+</sup>Ly6G<sup>+</sup> population was identified as granulocytic MDSCs (G-MDSCs).

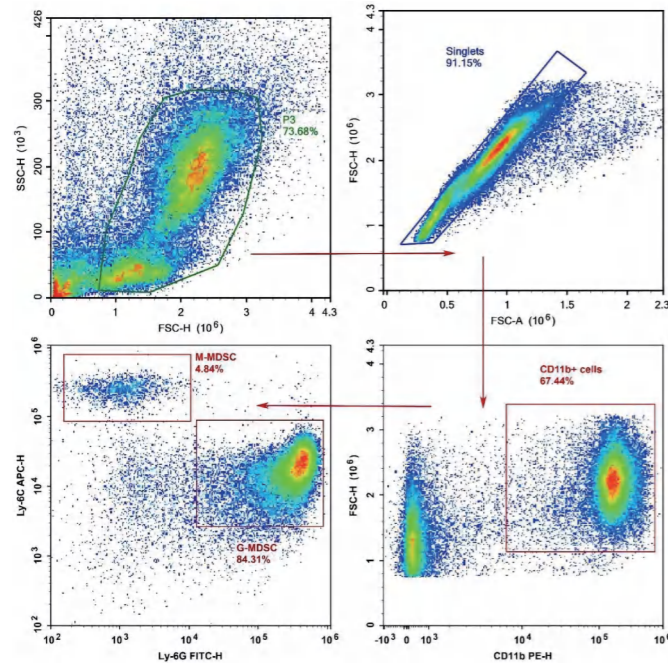
**Detection of MDSC (3-Color) in Mouse Bone Marrow (Panel 2)**

**01 Panel Design**

| Purpose   | Sample | Antibody Collocation                                   |
|---|--------|--|
| Adjust the voltage  | 1      | Blank  |
| Adjust compensation   | 2      | CD11b-PE   |
|   | 3      | Ly-6G-FITC   |
| PE-FMO in combination with Isotype Control for auxiliary gating   | 4      | Ly-6C-APC  |
|   | 5      | Ly-6G-FITC, Ly-6C-APC, Rat IgG2b, κ Isotype Control-PE |
| FITC-FMO in combination with Isotype Control for auxiliary gating | 6      | CD11b-PE, Ly-6C-APC, Rat IgG2a, κ Isotype Control-FITC |
| APC-FMO in combination with Isotype Control for auxiliary gating  | 7      | CD11b-PE, Ly-6G-FITC, Rat IgG2a, κ Isotype Control-APC |
| Full Panel  | 8      | CD11b-PE, Ly-6G-FITC, Ly-6C-APC                        |

**02 Information of Flow Cytometry Antibodies**

| Marker                      | Fluorochrome | Clone No. | Cat. No.     |
|-----------------------------|--------------|-----------|--------------|
| CD11b                       | PE           | M1/70     | E-AB-F1081D  |
| Ly-6G                       | FITC         | 1A8       | E-AB-F1108C  |
| Ly-6C                       | APC          | Monts 1   | E-AB-F1121E  |
| Rat IgG2b,κ Isotype Control | PE           | LTF-2     | E-AB-F09842D |
| Rat IgG2a,κ Isotype Control | FITC         | 2A3       | E-AB-F09832C |
| Rat IgG2a,κ Isotype Control | APC          | 2A3       | E-AB-F09832E |



### Tips:

- ⊞ The granulocytic MDSC (G-MDSC) phenotype is defined as  $CD11b^+Ly-6C^{low}Ly-6G^+$ , whereas the monocytic MDSC (M-MDSC) phenotype is  $CD11b^+Ly-6C^{high}Ly-6G^-$ .
- ⊞ In this experiment, CD11b-positive cells were gated to analyze the expression of Ly-6C and Ly-6G. Granulocytic MDSCs (G-MDSCs) and monocytic MDSCs (M-MDSCs) were distinguished based on their characteristic high expression of Ly-6G and Ly-6C, respectively.

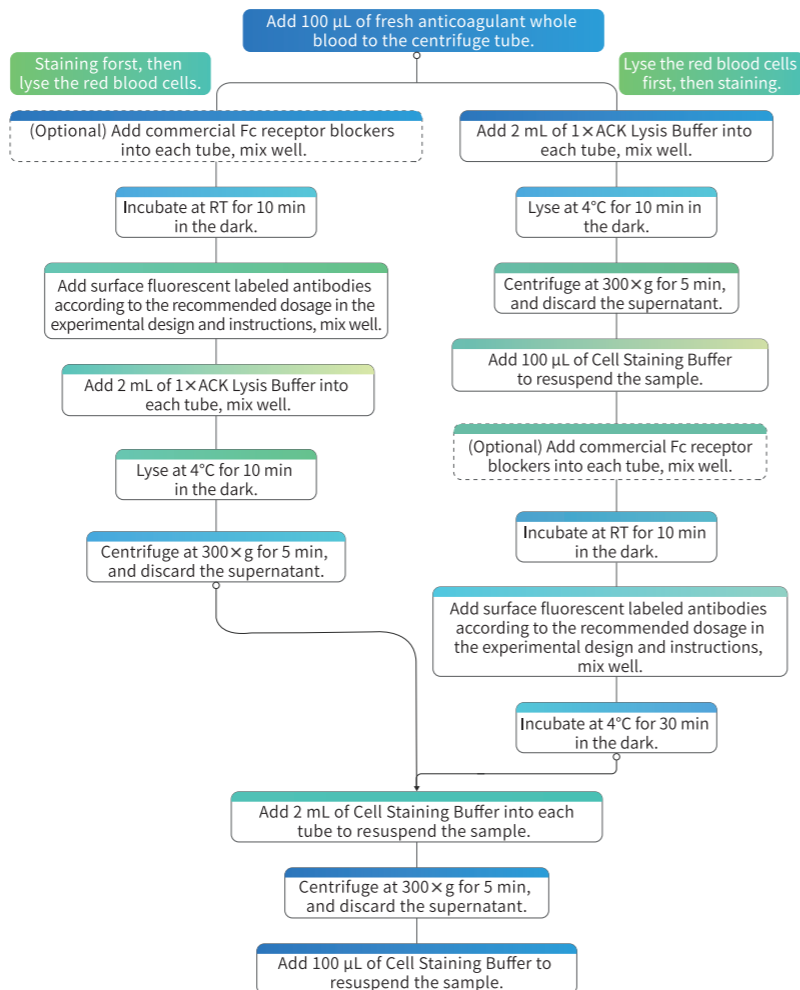
## Staining Protocol of Flow Cytometry

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

The staining process of Flow Cytometry 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 Flow Cytometry Staining Protocol

### 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. (For detailed protocols, please refer to the Elabscience® Flow Cytometry Sample Preparation Guide or visit the website: [www.elabscience.com](http://www.elabscience.com)).
2. After counting the suspension with a hemocytometer or other instruments, adjust the cell concentration to about  $1 \times 10^7$ /mL.
3. Add 100 µL 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 human cells**, EasyStain™ Human Fc Receptor Blocking Solution (**E-CK-A171**) can be used as an FcR blocking reagent. Add 5 µL of EasyStain™ Human Fc Receptor Blocking Solution, mix well, and incubate at room temperature for 10 min.
  - For mouse cells**, Purified Anti-Mouse CD16/CD32 Antibody specific for FcγR 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. Add 0.5-1 µg of Purified Anti-Mouse CD16/32 Antibody (**E-AB-F0997A**) and incubate at room temperature for 10 min.
  - For rat cells**, 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.
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×g for 5 min, and discard the supernatant.
7. Add 200 µL of Cell Staining Buffer to resuspend the sample, then detect by Flow Cytometer.

## Cell Intracellular Flow Cytometry Staining Protocol

1. Prepare the experimental sample (spleen, bone marrow, lymph nodes, thymus, etc.) into a single-cell suspension (For detailed protocols, please refer to the Elabscience® Flow Cytometry Sample Preparation Guide or visit the website: [www.elabscience.com](http://www.elabscience.com)).
2. Count the suspension with a hemocytometer or other instruments, adjust the cell concentration to about  $1 \times 10^7$ /mL.
3. Add 100  $\mu$ 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 human cells,** EasyStain™ Human Fc Receptor Blocking Solution (E-CK-A171) can be used as an FcR blocking reagent. Add 5  $\mu$ L of EasyStain™ Human Fc Receptor Blocking Solution, mix well, and incubate at room temperature for 10 min.

**For mouse cells,** 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. Add 1  $\mu$ g of Purified Anti-Mouse CD16/32 Antibody (E-AB-F0997A) and incubate at room temperature for 10 min.

**For rat cells,** 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.

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 2 mL of Cell Staining Buffer to resuspend cells. Centrifuge at 300 $\times$ g for 5 min, and discard the supernatant.
8. Add 200  $\mu$ L of Cell Staining Buffer to resuspend the sample. Add 200  $\mu$ L of 1 $\times$ Fixation Buffer to each tube, mix gently. Incubate at RT for 30-60 min in the dark.
9. Add 1 mL of 1 $\times$ Permeabilization Working Solution into each tube, mix gently. Centrifuge at 600 $\times$ g for 5 min, and discard the supernatant.
10. Add 100  $\mu$ L of 1 $\times$ 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  $\mu$ L of Cell Staining Buffer to resuspend the sample, detect by Flow Cytometer.

## Cell Intranuclear Flow Cytometry Staining Protocol

1. Prepare the experimental sample (spleen, bone marrow, lymph nodes, thymus, etc.) into a single-cell suspension. (For detailed protocols, please refer to the Elabscience® Flow Cytometry Sample Preparation Guide or visit the website: [www.elabscience.com](http://www.elabscience.com)).
2. Count the suspension with a hemocytometer or other instruments, adjust the cell concentration to about  $1 \times 10^7$ /mL.
3. Add 100  $\mu$ 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 human cells,** For human cells, EasyStain™ Human Fc Receptor Blocking Solution (**E-CK-A171**) can be used as an FcR blocking reagent. Add 5  $\mu$ L of EasyStain™ Human Fc Receptor Blocking Solution, mix well, and incubate at room temperature for 10 min.

**For mouse cells,** 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. Add 1  $\mu$ g of Purified Anti-Mouse CD16/32 Antibody (**E-AB-F0997A**) and incubate at room temperature for 10 min.

**For rat cells,** 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.

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  $300 \times g$  for 5 min, and discard the supernatant. Then add 100  $\mu$ L of Cell Staining Buffer to resuspend the cells.
8. Prepare 1 $\times$  Fixation and Permeabilization Buffer (It is recommended to use **E-CK-A108**):

**1  $\times$  Fixation Working Solution:** Dilute Fixation Concentrate (4 $\times$ ) with Fixation Dilution Solution to 1 $\times$  Fixation Working Solution.

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

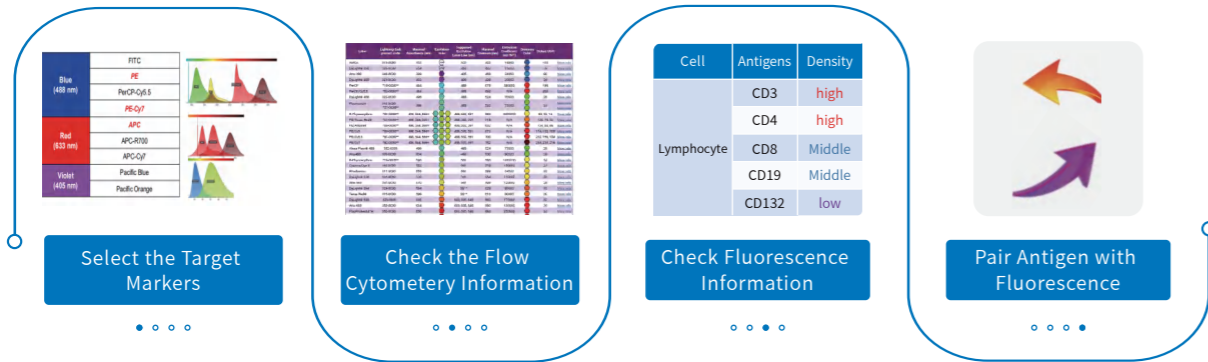
9. Add 1 mL of 1 $\times$  Fixation Working Solution to each tube and mix well, incubate the cells at 4°C for 30 min, then centrifuge at  $600 \times g$  for 5 min and discard the supernatant.
10. Add 2 mL of 1 $\times$  Permeabilization Working Solution to each tube and mix well, centrifuge at  $600 \times g$  for 5 min and discard the supernatant.
11. Repeat Step 10.
12. Resuspend the cells with 100  $\mu$ L of 1 $\times$  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 $\times$  Permeabilization Working Solution to each tube and centrifuge at  $600 \times g$  for 5 min at RT. Discard the supernatant.
14. Add 200  $\mu$ L of Cell Staining Buffer to resuspend the sample, detect by Flow Cytometer.

# Elabscience® Featured Services of Flow Cytometry

## 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.

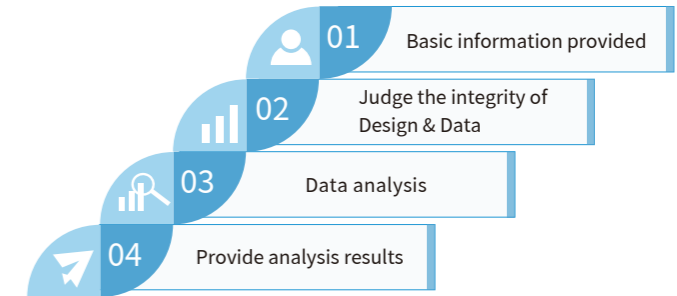
### Process of Panel Design Services



## 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



## Product Citations

Trusted by 28,500+ SCI publications, continually empowering scientific research.

| Product Name                           | Cat. No.     | Citation  | IF   |
|--|--------------|---|------|
| PE Anti-Mouse CD119 Antibody[GR-20]    | E-AB-F1115D  | Benguigui M, Cooper T J, Kalkar P, et al. Interferon-stimulated neutrophils as a predictor of immunotherapy response[J]. <i>Cancer Cell</i> , 2024, 55 1.                           | 48.8 |
| PerCP Anti-Mouse CD48 Antibody[HM48-1] | E-AB-F1017UF | Simats A, Zhang S, Messerer D, Chong F, et al. Innate immune memory after brain injury drives inflammatory cardiac dysfunction[J]. <i>Cell</i> . 2024 Aug 22;187(17):4637-4655.e26. | 45.5 |
| PE Anti-Mouse CD54 Antibody[YN1/1.7.4] | E-AB-F1018D  | Born E, Lipskaia L, Breau M, et al. Eliminating senescent cells can promote pulmonary hypertension development and progression[J]. <i>Circulation</i> , 2023, 147(8): 650-666.      | 37.8 |
| APC Anti-Mouse CD8a Antibody[53-6.7]   | E-AB-F1104E  | Lu C, Liao S, Chen B, et al. Responsive probes for in vivo magnetic resonance imaging of nitric oxide[J]. <i>Nature Materials</i> , 2024, 1-10.                                     | 37.2 |
| PE Anti-Mouse CD25 Antibody[PC-61.5.3] | E-AB-F1102D  |   |      |
| FITC Anti-Mouse Foxp3 Antibody[3G3]    | E-AB-F1238C  |   |      |
| PE Anti-Mouse F4/80 Antibody[Cl:A3-1]  | E-AB-F0995D  |   |      |
| Biotin Anti-Mouse CD31 Antibody[390]   | E-AB-F1180B  |   |      |
| APC Anti-Mouse Ly6C Antibody[Monts 1]  | E-AB-F1121E  |   |      |
|  |              |   |      |

| Product Name  | Cat. No.     | Citation  | IF   |
|---|--------------|---|------|
| Purified Anti-Mouse CD16 /32 Antibody[2.4G2]          | E-AB-F0997A  | Lu C, Liao S, Chen B, et al. Responsive probes for in vivo magnetic resonance imaging of nitric oxide[J]. <i>Nature Materials</i> , 2024, 1-10.   | 37.2 |
| APC Anti-Mouse Ly-6G/ Ly-6C (Gr-1) Antibody [RB6-8C5] | E-AB-F1120UE |   |      |
| PE Anti-Mouse CD54 Antibody[YN1/1.7.4]                | E-AB-F1018D  | Che Y J, Ren X H, Wang Z W, et al. Lymph-Node-Targeted Drug Delivery for Effective Immunomodulation to Prolong the Long-Term Survival After Heart Transplantation[J]. <i>Advanced Materials</i> , 2022            | 32.0 |
| PE/Cyanine7 Anti-Mouse CD11c Antibody[N418]           | E-AB-F0991UH | Yin D, Zhong Y, Ling S, et al. Dendritic-cell-targeting virus-like particles as potent mRNA vaccine carriers[J]. <i>Nature Biomedical Engineering</i> , 2024, 1-16.   | 28.1 |
| FITC Anti-Mouse Ly6G Antibody[1A8]                    | E-AB-F1108C  | Cai J, Quan Y, Zhu S, et al. The browning and mobilization of subcutaneous white adipose tissue supports efficient skin repair[J]. <i>Cell Metabolism</i> , 2024, 36(6), 1287-1301.                               | 27.7 |
| FITC Anti-Mouse CD3 Antibody[17A2]                    | E-AB-F1013C  | Mai Z, Fu L, Su J, et al. Intra-tumoral sphingobacterium multivorum promotes triple-negative breast cancer progression by suppressing tumor immunosurveillance [J]. <i>Molecular Cancer</i> . 2025 Jan 8;24(1):6. | 27.7 |
| PE/Cyanine7 Anti-Mouse CD4 Antibody[GK1.5]            | E-AB-F1097H  |   |      |
| PE Anti-Mouse CD25 Antibody[PC-61.5.3]                | E-AB-F1102D  |   |      |
| PE Anti-Mouse CD206/ MMR Antibody[C068C2]             | E-AB-F1135D  | Y. Shuai, T. Yang, M. Zheng, et al.. Yang, Oriented Cortical-Bone-Like Silk Protein Lamellae Effectively Repair Large Segmental Bone Defects in Pigs[J]. <i>Advanced Materials</i> . 2025, 37, 2414543.           | 27.4 |

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