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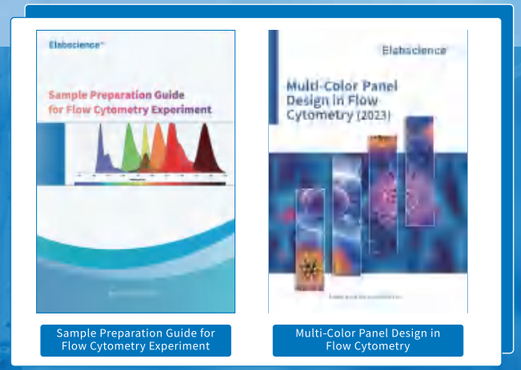


Sample Preparation Guide for Flow Cytometry Experiment

Multi-Color Panel Design in Flow Cytometry


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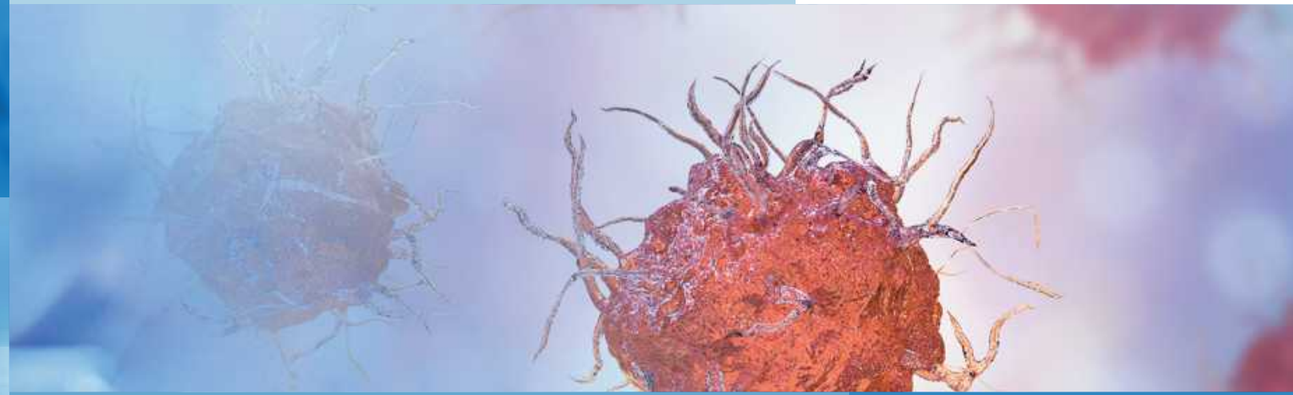
# Guide to Phenotyping Immune Cells Using a Flow Cytometer

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



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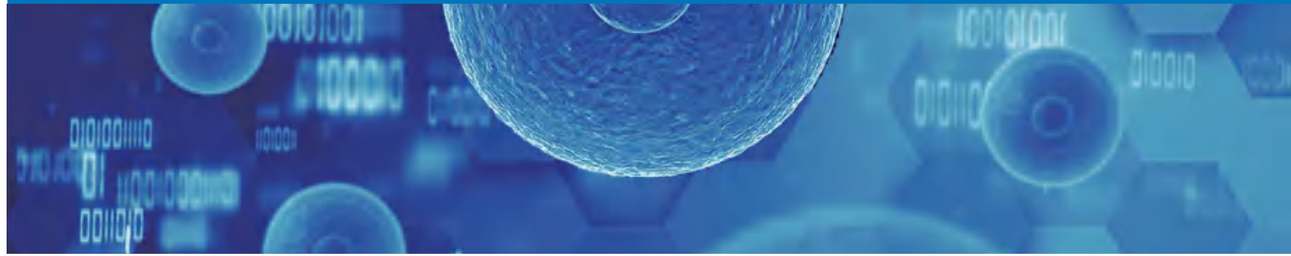
# About Elabscience®


Elabscience® empowers researchers in Cell Detection Areas by providing high-quality reagents and services. We have complete platform for R&D and manufacture. At the same time, we have in house QC for every product, endeavoring to keep your experiment results more consistent and precise. Through unremitting effort and development, our customers have spread in more than 150 countries and regions worldwide.

Elabscience® major products cover ELISA Kits, CLIA Kits, FCM Antibodies, Cell Culture Products, Cell Apoptosis Assay Kits, Cell Metabolism Assay Kits, Recombinant Proteins, Antibodies, Food Safety Kits and Other Reagents
Elabscience® also offers custom services for our customers including Protein Service, Polyclonal Antibody Service, Peptide Service and Gene Synthesis.



# CONTENTS

# 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 | • Participate in cellular immunity and directly kill pathogen cells.<br>• Release some lymphatic factors to enhance the immune response of the body. |
| B lymphocytes | • Participate in humoral immunity and produce antibodies.<br>• Present soluble antigens.<br>• Produce cytokines involved in immune regulation. |
| NK cells | • Killing target cells such as viral infections or tumors.<br>• Secreting cytokines participates in immune regulation. |

Table 1: Function of different lymphocytes

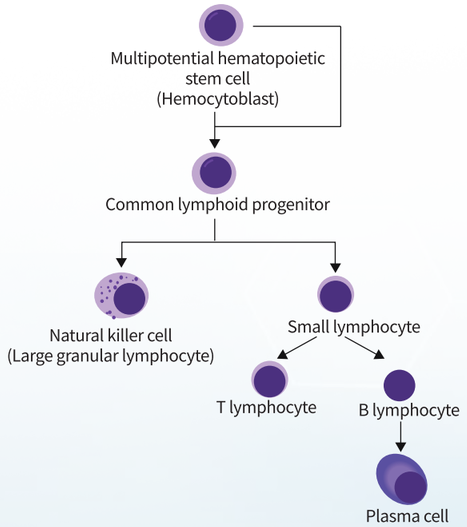



Fig 1: Differentiation of lymphocytes

*(Image from Medical Immunology)*



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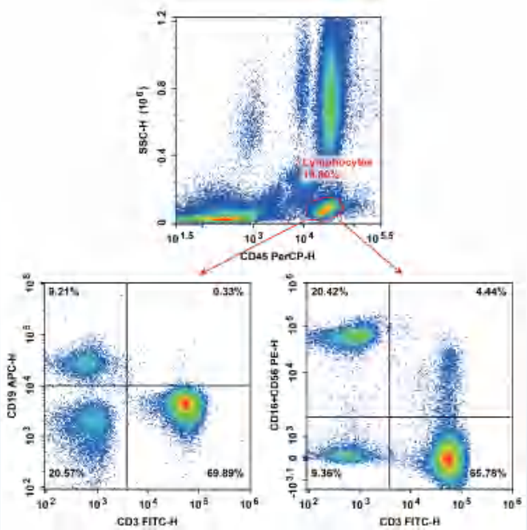
## Detection of T/B/NK (4-color) in Human Peripheral Blood

### 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 |
| | 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 |




**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 $CD3^{-}CD16^{+}$ $CD56^{+}$.
- 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.



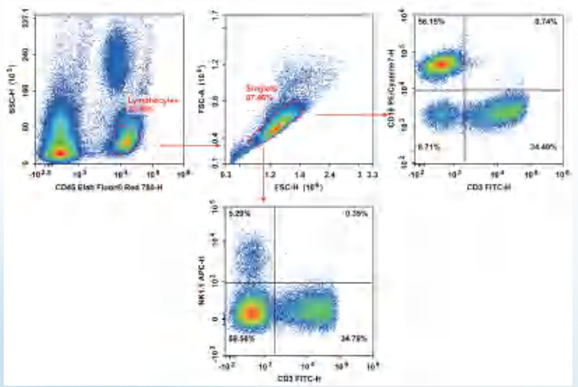
# Detection of T/B/NK (4-color) in C57BL/6 Mouse Peripheral Blood

## 01 Panel Design

| Purpose | Sample | Antibody Collocation |
|---|---|---|
| Adjust the voltage | 1 | Blank |
| Adjust compensation | 2 | CD45-Elab Fluor® Red 780 |
| | 3 | CD3-FITC |
| | 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). $CD3^-NK1.1^+/CD3^-CD49b^+$ 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.





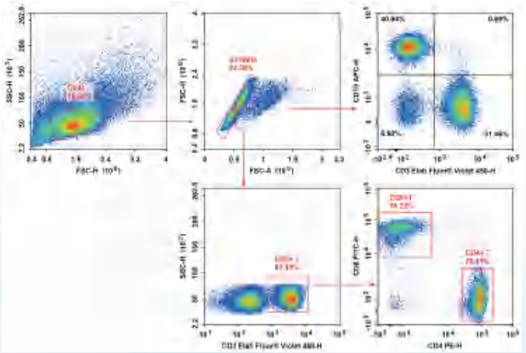
## Detection of TB cells (4-color) in Mouse Lymph Node

### 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.

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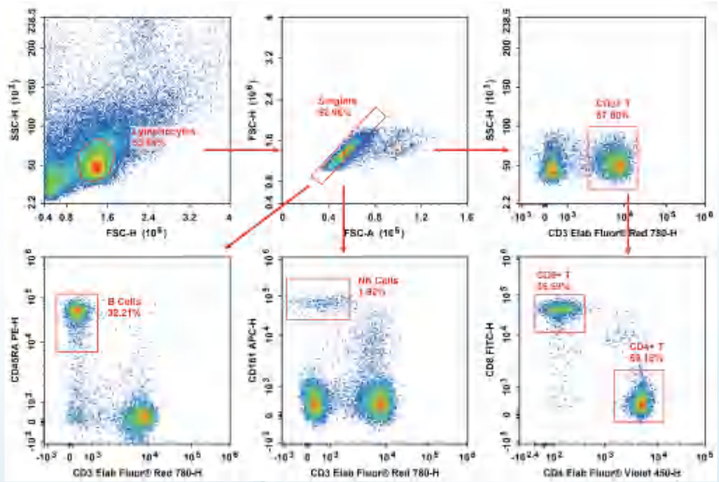
# Detection of T/B/NK (5-color) in Rat Spleen

## 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 $CD3^-CD45RA^+$, and the phenotype of NK cells is $CD3^-CD161^+$.

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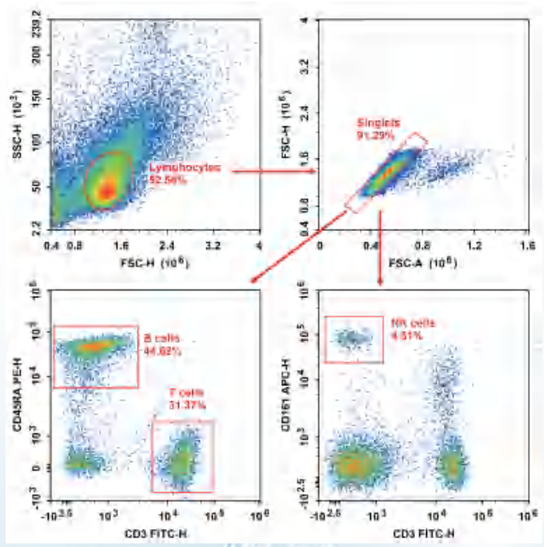
# Detection of T/B/NK (3-color) in Rat Spleen

## 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 $CD3^{-}CD45RA^{+}$, and the phenotype of NK cells is $CD3^{-}CD161^{+}$.
- Isotype Control for CD45RA and CD161 is suggested for auxiliary gating.

# CD4⁺/CD8⁺ T Cell Population Detection

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

- **CD4⁺T cell:** CD4⁺ 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⁺T cell:** CD8⁺ 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.

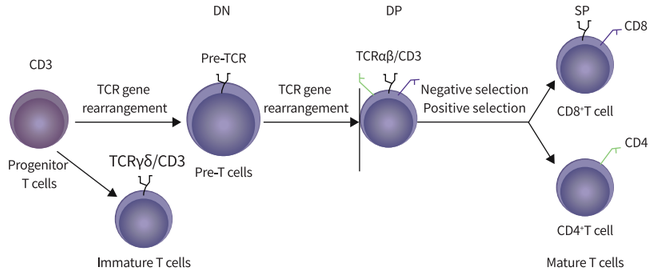



Fig 2: The developmental process of T cells in the thymus

*(Image from Medical Immunology)*

DN: Double negative ($CD4^-CD8^-$);

DP: Double positive ($CD4^+CD8^+$);

SP: Single positive ($CD4^+CD8^-$ or $CD4^-CD8^+$).





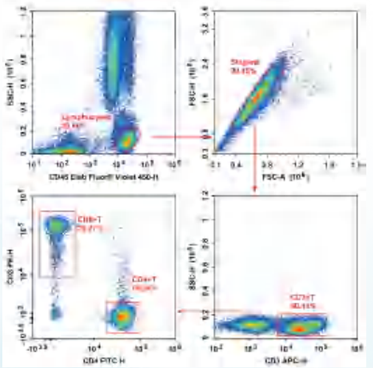
## Detection of T Cells (4-color) in Human Peripheral Blood

### 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 |



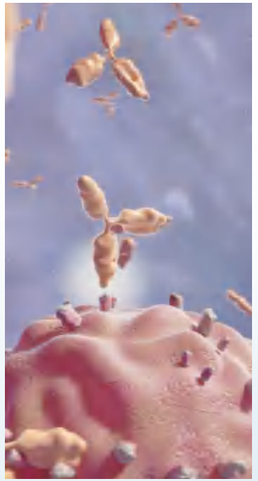
**Tips:**

- 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.

# 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, κ Isotype Control-APC |
| FITC-FMO in combination with Isotype Control for auxiliary gating | 3 | CD8a-APC; Rat IgG2b, κ Isotype Control- FITC |
| Full Panel | 4 | CD4-FITC, CD8a-APC |

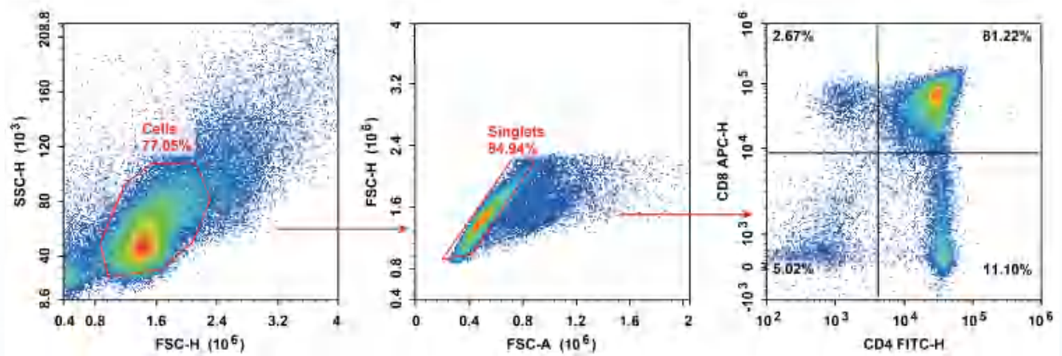


## 02 Information of Flow Cytometry Antibodies

| Marker | Fluorochrome | Clone No. | Cat. No. |
|---|---|---|---|
| CD4 | FITC | GK1.5 | E-AB-F1097C |
| CD8a | APC | 53-6.7 | E-AB-F1104E |
| Rat IgG2b, κ Isotype Control | FITC | LTF-2 | E-AB-F09842C |
| Rat IgG2a, κ 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: $CD4^-CD8^-$), double positive cells (DP cells: $CD4^+CD8^+$) and single positive cell (SP cell: $CD4^+CD8^-$ or $CD4^-CD8^+$).
- 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.

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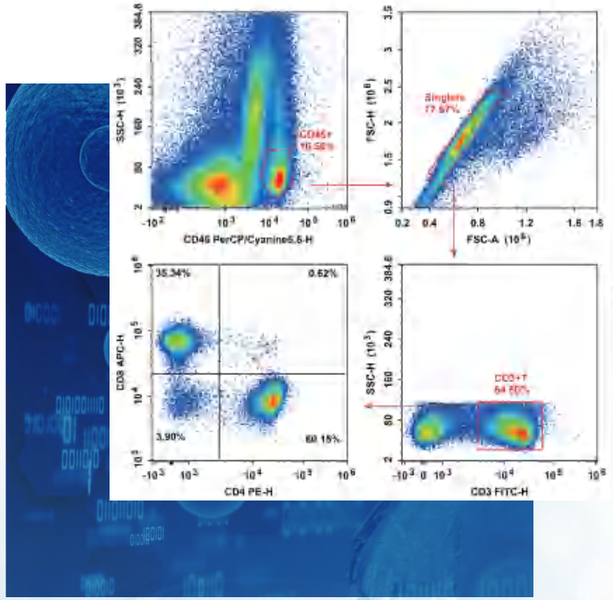
# Detection of Lymphocyte (4 colors) in Mouse Tumor

## 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 $CD45^{high}$ and $SSC^{low}$ 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.

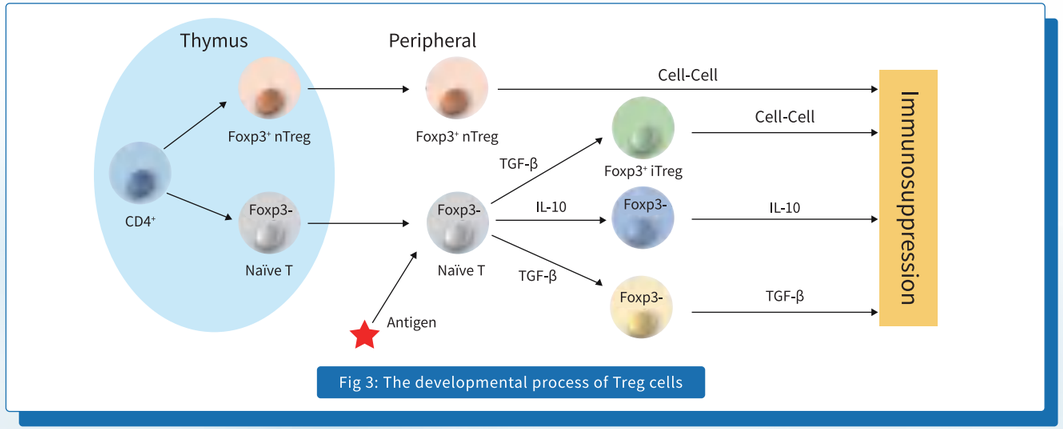



Fig 3: The developmental process of Treg cells





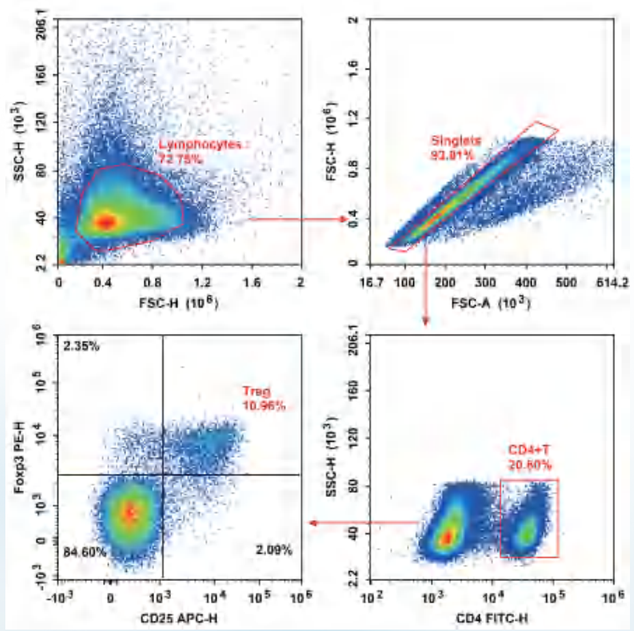
# Detection of Treg (3-color) in Mouse Spleen

## 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 $CD4^+$ $CD25^+$ $Foxp3^+$.
- 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.





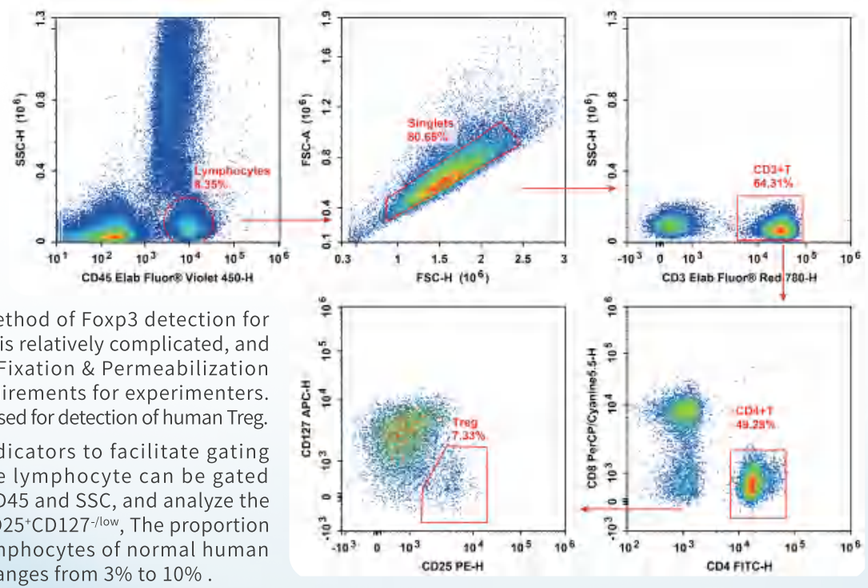
## Detection of Treg (6-color) in Human Peripheral Blood

### 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 $CD4^+CD25^+CD127^{-/low}$, 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.

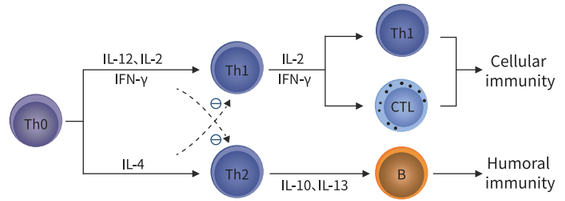



Fig 4: The regulatory effect of cytokines on Th1 and Th2 cell subpopulations

*(Image from Medical Immunology)*

- **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.

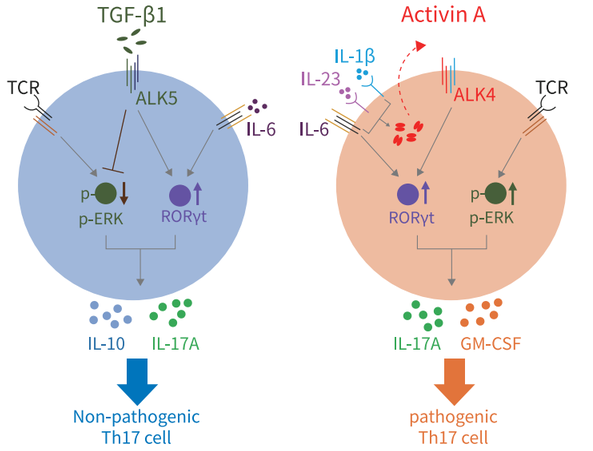



Fig 5: Differentiation and function of Th17 cells

(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

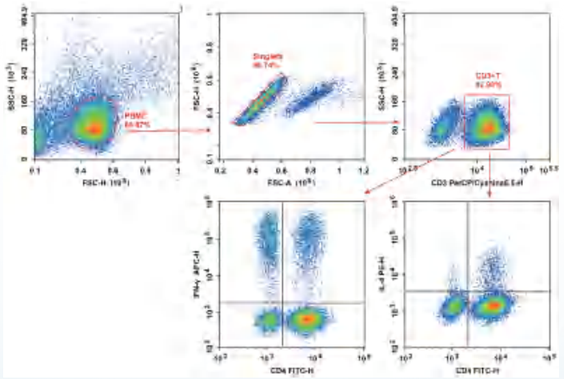
| Purpose | Sample | Antibody Collocation |
|---|---|---|
| Adjust the voltage | 1 | Blank |
| Adjust compensation | 2 | CD3-PerCP/Cyanine5.5 |
| | 3 | CD4-FITC |
| | 4 | IFN-γ-APC |
| | 5 | IL-4-PE |
| APC-FMO in combination with Isotype Control for auxiliary gating | 6 | CD3-PerCP/Cyanine5.5, CD4-FITC, 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-γ and IL-4 are necessary, since the expression of cytokines is generally not high.



- $CD3^+$ $CD4^+$ IFN-$\gamma^+$ is Th1 type, $CD3^+$ $CD4^+$ IL-$4^+$ 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.





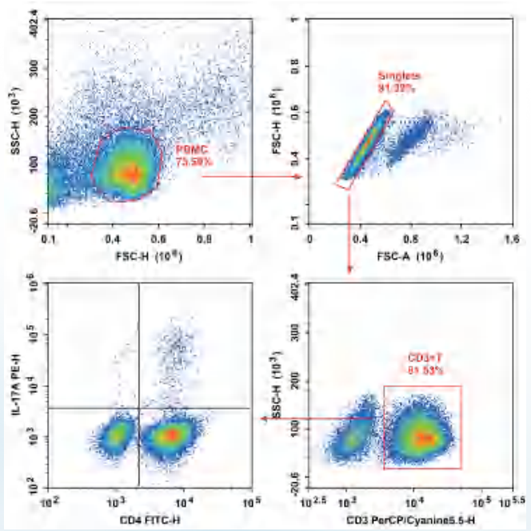
# Detection of Th17 (3-color) in Human PBMC

## 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, 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 | UCHT1 | E-AB-F1230J |
| CD4 | FITC | SK3 | E-AB-F1352C |
| 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 (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.
- $CD3^+CD4^+$ $IL\text{-}17A^+$ 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.





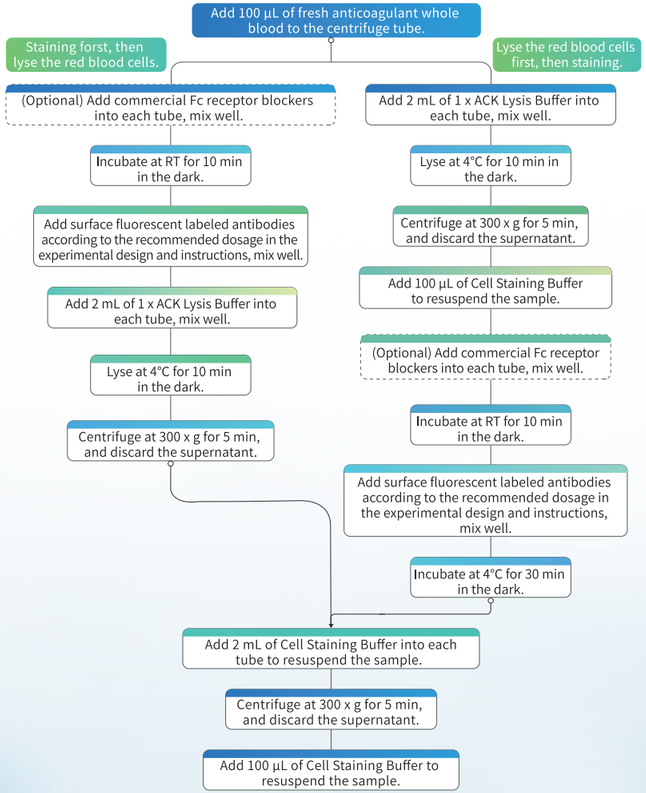
# Flow Cytometry Staining Protocol

- 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 x $10^7$/mL.
3. Add 100 µL of cell suspension (approximately 1 x $10^6$ 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 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. 1 µg 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 µg 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 µL of Cell Staining Buffer to resuspend the sample, detect by Flow Cytometer.

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## 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 1~2 x $10^6$/mL.
2) Add 2 µL of 500 x Cell Stimulation MIX to each 1 mL of cell suspension, and incubate the cells at 37°C, 5%$CO_2$ for 4~18 h.
3) Add 1 µL of 1000 x Protein Transport Inhibitor MIX to each 1mL of cell suspension, and incubate the cells at 37°C, 5%$CO_2$ for 5~16 h.
4) Collect cell suspension, centrifuge at 200~300 x 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 x $10^7$/mL.
3. Add 100 µL of cell suspension (approximately 1 x $10^6$ 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 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. 1 µg 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 µg of purified anti-human CD16 monoclonal antibody (E-AB-F1236A) was added and incubated at room temperature for 10 min.

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 x g for 5 min, and discard the supernatant.
8. Add 200 µL of Cell Staining Buffer to resuspend the sample. Add 200 µL 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 x g for 5 min, and discard the supernatant.
10. Add 100 µL 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 x g for 5 min and discard the supernatant.
12. Add 200 µL of Cell Staining Buffer to resuspend the sample, detect by Flow Cytometer.

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## 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 x $10^7$/mL.
3. Add 100 µL of cell suspension (approximately 1 x $10^6$ 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 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. 1 µg 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 µg of purified anti-human CD16 monoclonal antibody (E-AB-F1236A) was added and incubated at room temperature for 10 min.

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 µL 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 $ddH_2O$ to 1x Permeabilization Working Solution.

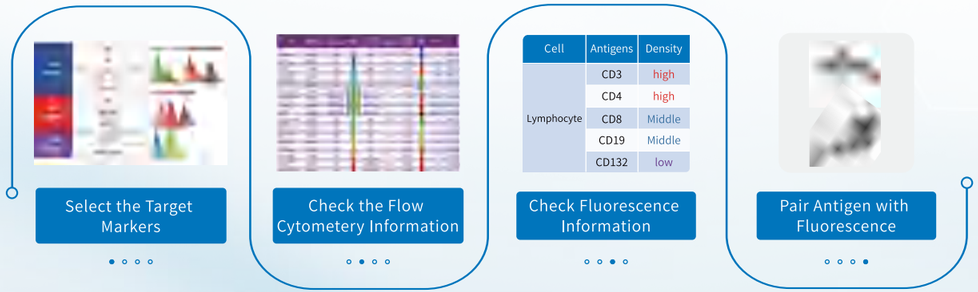
9. Add 1 mL of 1 x Fixation Working Solution to each tube and mix well, incubate the cells at 4°C for 30 min, then centrifuge at 600 x g for 5 min and discard the supernatant.
10. Add 2 mL of 1 x Permeabilization Working Solution to each tube and mix well, centrifuge at 600 x g for 5 min and discard the supernatant.
11. Repeat Step 10.
12. Resuspend the cells with 100 µL 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 µL 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