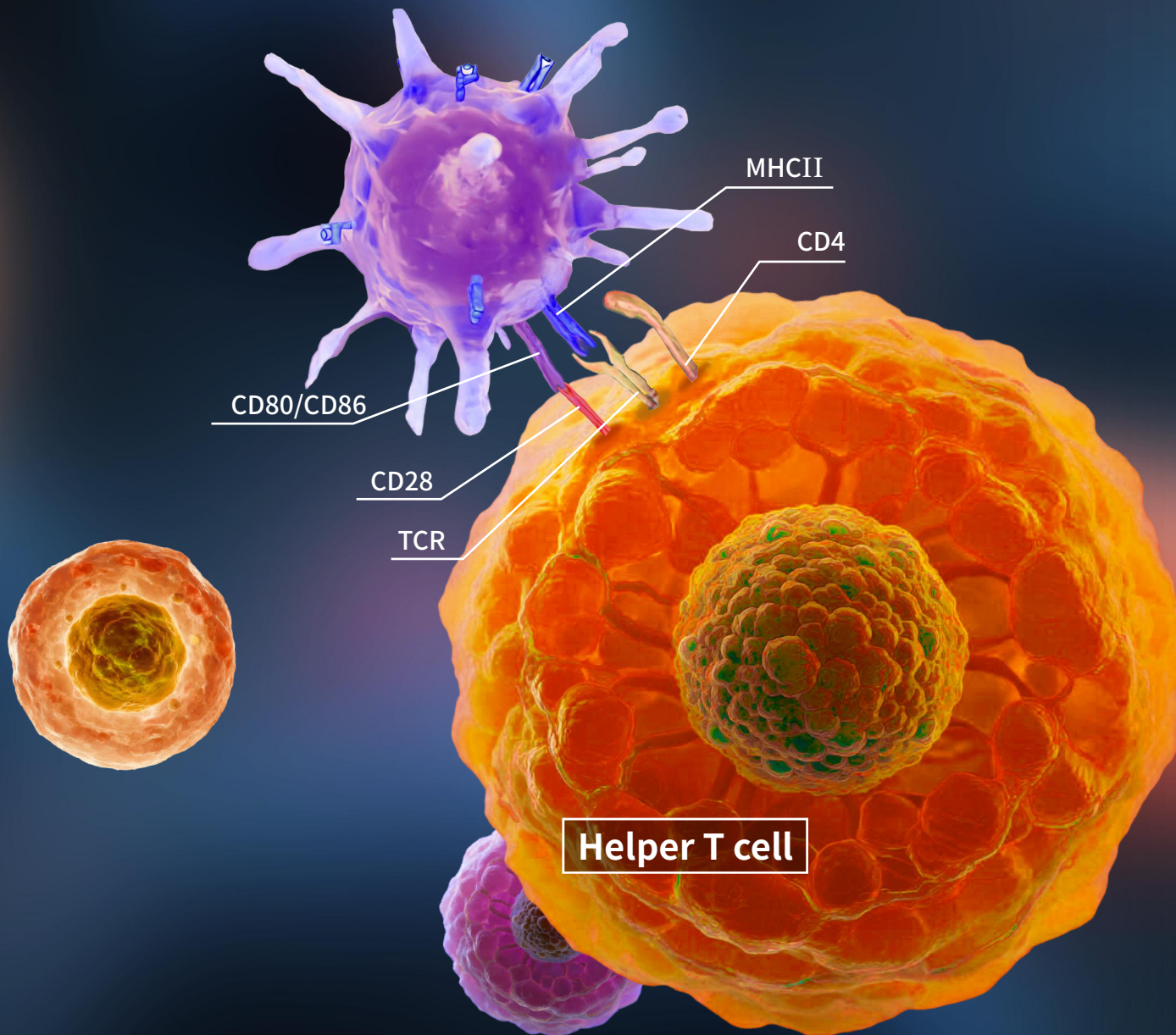


Elabscience®

# One-stop Solution for T Cell Research



## Expertise in Cell Studies, Providing One-stop Solution

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 assays, cell cycle assays, cell proliferation/cytotoxicity/viability).

Our relentless pursuit of excellence since 2011 has established our presence in over 150 countries and regions globally.



ISO9001:2015  
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System Certification



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2025

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Technical  
Platforms

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# 01 Introduction to T Cells

## ■ T Cell Development

T lymphocytes originate from common lymphoid progenitors, which are derived from hematopoietic stem cells in the bone marrow. These precursor cells migrate to the thymus via the bloodstream. In the thymic microenvironment, they undergo complex differentiation and selection processes, and finally mature into immunocompetent T cells.

In the thymus, immature T cells must go through strict screening mechanisms (such as positive selection and negative selection) to ensure that T cells respond only to pathogens or abnormal cells and avoid attacking the body's own tissues. In the thymus, T cells go through the developmental stages of double-negative (DN) → double-positive (DP) → single-positive (SP):

### Double-negative Stage (DN)

The cells do not express CD4/CD8 on the cell surface. Development is initiated through the rearrangement of the TCR β chain. The cells then go through the DN1-DN4 sub-stages and finally form a functional TCR β chain.

### Double-positive Stage (DP)

Cells express both CD4 and CD8 simultaneously. The MHC restriction of TCR is determined through positive selection (by binding to the self-major histocompatibility complex MHC of thymic cortical epithelial cells). Cells that fail to pass the selection undergo apoptosis.

### Single-positive Stage (SP)

Cells differentiate into CD4<sup>+</sup> T cells (which bind to MHC class II molecules) or CD8<sup>+</sup> T cells (which bind to MHC class I molecules). Self-reactive T cells are eliminated through negative selection to establish peripheral tolerance.

Once T cells mature in the thymus, they migrate and settle in the peripheral lymphoid organs of the immune system, such as lymph nodes and the spleen. They then circulate throughout the body via the lymphatic system, always ready to respond to the invasion of foreign antigens.

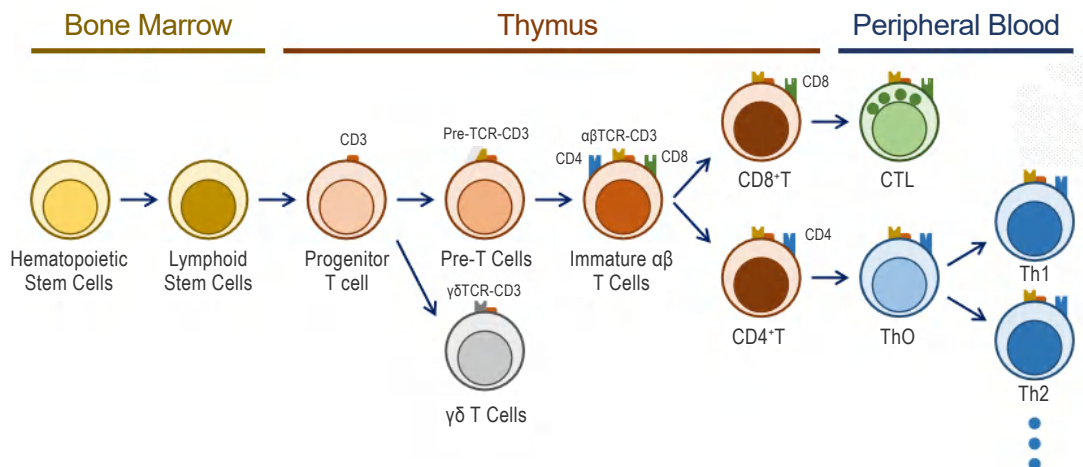


Fig. 1. Differentiation and development of T cells.

## ■ T Cell Classification

As one of the most important adaptive immunities, T cells serve as the body's natural defense against cancer and infectious diseases. As such, T cells are a type of lymphocytes that consist of multiple cell subsets and possess various biological functions. Common T cell subsets include CD4<sup>+</sup> T cells, CD8<sup>+</sup> T cells, Helper T cells (Th), Regulatory T cells (Tregs), Cytotoxic T cells (Tcs), Naïve T cells, Effector T cells, and Memory T cells, etc. These T cell subsets are classified based on distinct criteria. Common classification criteria include:

### The Composition and Diversity of TCR Molecules

TCR is the core molecule for T cells to recognize antigens and also the marker molecule for defining T cells. The TCR molecule is generally composed of two different subunits, and each subunit contains a variable region. There are differences in the composition and structure of TCR molecules among different T cells. Based on this, T cells can be divided into classic T cells and non-classic T cells ( $\gamma\delta$  T cells, NKT cells, and MAIT cells):

- **Classic T Cells:** The TCR is composed of an  $\alpha$ -chain and a  $\beta$ -chain. Both chains are generated through gene rearrangement (random combination of V, D, and J segments), resulting in a highly diverse structure.
- **$\gamma\delta$  T Cells:** The TCR is composed of a  $\gamma$ -chain and a  $\delta$ -chain. The gene rearrangement is simple, and the diversity is far lower than that of  $\alpha\beta$  TCR. They recognize non-peptide antigens presented by non-classic MHC molecules of the CD1 family.
- **NKT Cells:** They express a "semi-invariant TCR" (such as Va14-Ja18 in mice and Va24-Ja18 in humans), with almost no diversity. They recognize lipid and glycolipid antigens presented by non-classical MHC class I-like molecule of the CD1 family.
- **MAIT Cells:** The TCR is mainly Va7.2-Ja33 (in humans), with a constant structure and extremely low diversity. They mainly recognize bacterial metabolites and guard the mucosal barrier.

### Activation State

- **Naïve T Cells:** A mature T cell that has never been stimulated by an antigen. Naïve T cells have a low metabolic level and weak proliferative ability.
- **Activated T Cells:** After a Naïve T cell receives an activation signal (in the body, generally when the TCR recognizes the antigen-peptide-MHC complex presented by an antigen-presenting cell (APC) and the co-stimulatory signals (such as the combination of CD28-B7) interact), its metabolic level increases, it enters the cell proliferation stage, and further differentiates into different effector T cells during the proliferation process.
- **Effector T Cells:** After being stimulated by an antigen, a Naïve T cell proliferates and differentiates into a mature T cell that can perform effector functions, with functions include cytokine release (e.g., IFN- $\gamma$ ) and apoptosis (e.g., granzyme B).
- **Memory T Cells:** After being stimulated by an antigen, a T cell proliferates and differentiates into a cell that can survive for an extended duration and can respond quickly to the same antigen in a secondary immune response. According to different phenotypes and migration characteristics, memory T cells can be further divided into central memory T cells (TCM), effector memory T cells (TEM), tissue-resident memory T cells (TRM), and stem-cell-like memory T cells (TSCM).
- **T Cell Exhaustion:** In chronic infections or tumors, T cells are exposed to antigens for a long time and enter a functionally impaired exhausted state, losing effector functions and memory characteristics.

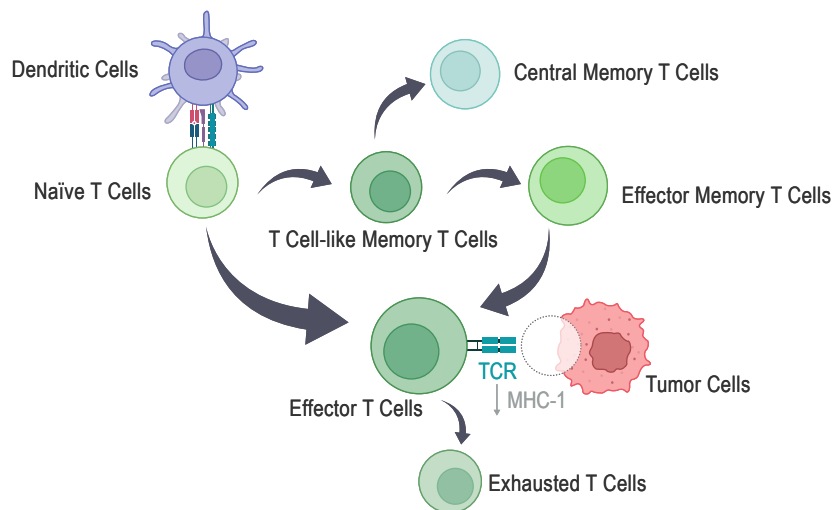


Fig. 2. T cell differentiation (DOI: 10.12354/j.issn.1000-8179.2023.20221449).

## Effector Functions (for Classical T Cells)

- **Helper T Cells (Th1, Th2, Th9, Th17, Th22, Tfh):** The "commanders" of the immune system, with the main surface marker CD4. They can secrete cytokines to activate B cells, macrophages, etc.
- **Cytotoxic T Cells (Tc1, Tc2, Tc9, Tc17):** The main surface marker is CD8. They directly kill target cells through perforin/granzyme and are the core combat force against tumors and viruses.
- **Regulatory T Cells (Tregs):** They inhibit excessive immune responses, maintain self-tolerance, prevent autoimmune diseases, and act as the "brake" of the immune system.

## T Cell and Diseases

As immune cells with immunomodulatory and cell-killing functions, the dysfunction of T cells will lead to the occurrence of various diseases. The immune system's recognition of "self" and "non-self" depends on the precise regulation of T cells. When this balance is disrupted, autoimmune diseases will occur. For example, in multiple sclerosis (MS), autoreactive CD4+ T cells (such as T cells targeting myelin basic protein) break through the blood-brain barrier, activate macrophages and microglia, damage the nerve myelin sheath, and cause nerve conduction disorders. T cell dysfunction is also one of the important driving forces for tumor occurrence and progression. Representative events include T cell exhaustion, massive infiltration of Treg cells, and metabolic inhibition of T cells by tumor cells.

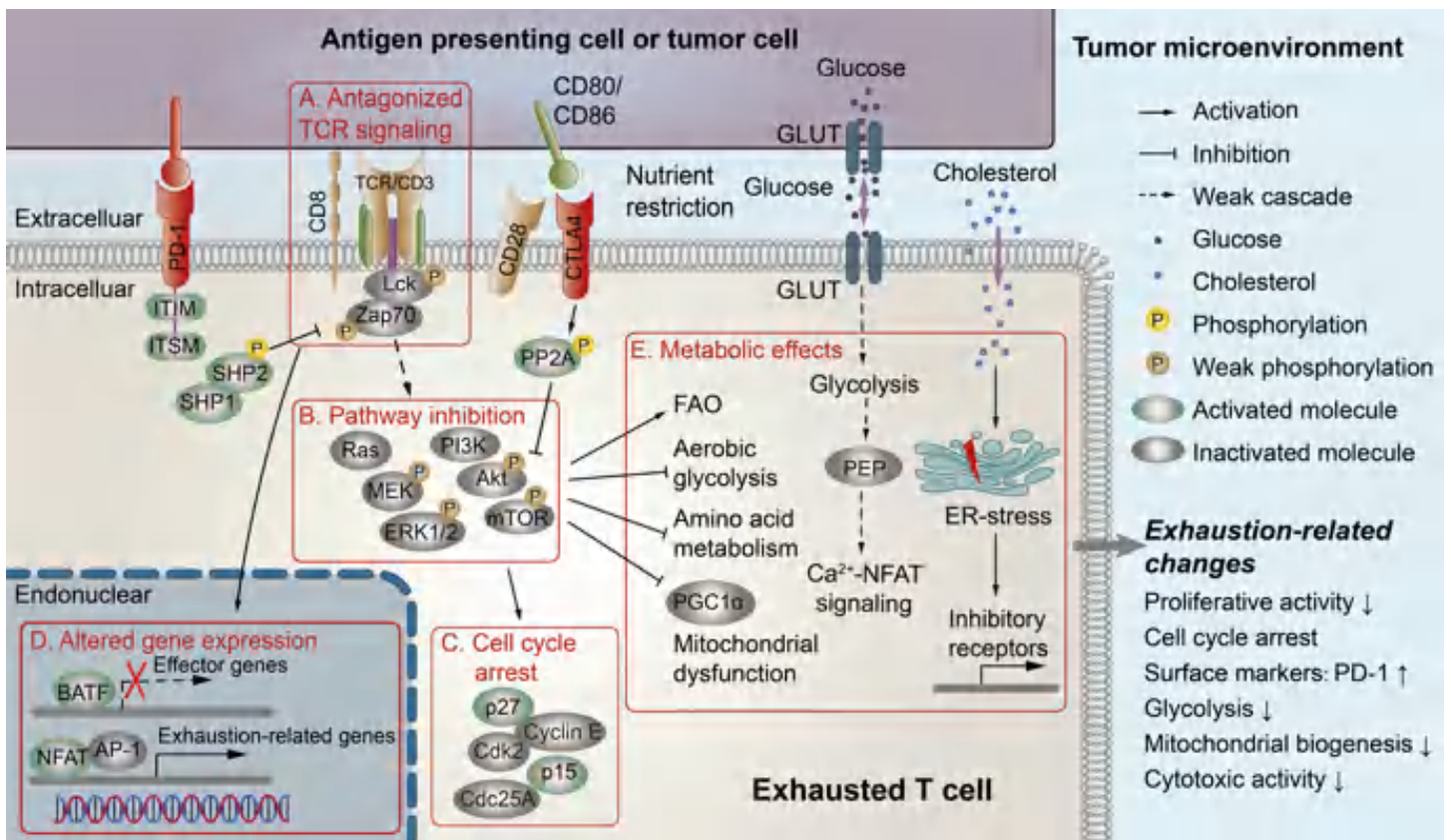


Fig. 3. PD-1 and CTLA4 signaling pathways and metabolic regulations involved in T-cell exhaustion in the tumor microenvironment. (DOI: 10.1038/s41423-019-0344-8).

Throughout the entire process of T cell related basic research and clinical translation, common experimental procedures include: T cell identification and typing, evaluation of effector T cell functions, determination of T cell metabolic status, and T cell isolation and culture. Elabscience® offers a full range of relevant products for T cell research, including: Flow Cytometry Antibodies, EasySort™ Cell Isolation Products (such as those for CD3, CD4, CD8 and memory T cells etc.), CD3/CD28 T Cell Activation Beads, ELISA Kits, as well as various kits for cell apoptosis assays, cell proliferation assays, cell cycle assays, and cell metabolism assays. In addition to products, Elabscience® has a professional technical team that provides customers with efficient and professional technical guidance, free flow cytometry panel design services, and data analysis services.

# 02 T Cell Isolation and Activation

In immunotherapy, CAR-T development, and basic scientific research, T cells with high purity and no activation interference are the key to the success of experiments. Traditional positive selection methods (such as CD3 magnetic bead labeling) may affect T cell receptor (TCR) signal transduction, leading to pre-activation or functional changes of T cells. Elabscience® provides negative selection kits for human and mouse T cells. This series of products uses magnetic bead negative selection technology, which does not require direct labeling of T cells. Moreover, they avoid cell activation or epitope masking caused by antibody binding, preserve the natural state of cells, and provide more reliable data support for functional experiments!

## ■ T Cell Isolation

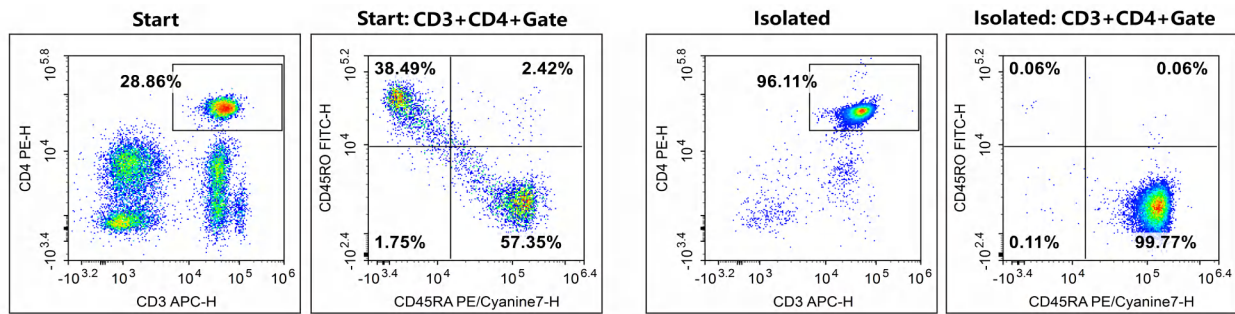


Fig. 4. Naïve CD4<sup>+</sup>T cells were isolated using the EasySort™ Human Naïve CD4<sup>+</sup>T Cell Isolation Kit (MIH007N).

Naïve CD4<sup>+</sup>T(CD3<sup>+</sup>CD4<sup>+</sup>CD45RO<sup>+</sup>/CD45RA<sup>-</sup>) cells were isolated from the Human PBMC, and were stained with APC Anti-Human CD3 Antibody[OKT-3] (E-AB-F1001E), PE Anti-Human CD4 Antibody[RPA-T4] (E-AB-F1109D), FITC Anti-Human CD45RO Antibody[UCHL1] (E-AB-F1139C) and PE/Cyanine7 Anti-Human CD45RA Antibody[HI100] (E-AB-F1052H). The purities of initial and final isolated fractions were 16.55% and 95.89%, respectively.

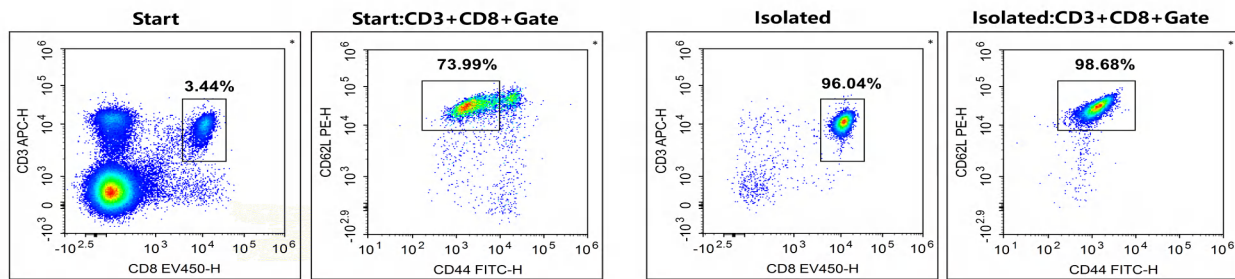


Fig. 5. Naïve CD8<sup>+</sup>T cells were isolated using the EasySort™ Mouse Naïve CD8<sup>+</sup>T Cell Isolation Kit (MIM008N).

Naïve CD8<sup>+</sup>T(CD3<sup>+</sup>CD8<sup>+</sup>CD44<sup>-/low</sup>CD62L<sup>high</sup>) cells were isolated from the spleen cells of C57BL/6 mice, and were stained with APC Anti-Mouse CD3ε Antibody[145-2C11] (E-AB-F1103E), Elab Fluor® Violet 450 Anti-Mouse CD8α Antibody[53-6.7](E-AB-F1104Q), FITC Anti-Mouse CD44 Antibody[NIM-R8](AN00917C) and PE Anti-Mouse CD62L Antibody[MEL-14](E-AB-F1011D). The purities of initial and final isolated fractions were 2.55% and 94.77%, respectively.

Product Name	Cat. No.
Human PBMC Separation Solution (P 1.077)	E-CK-A103
EasySort™-5 Magnet	EC001
EasySort™ Human Naïve Pan T Cell Isolation Kit	MIH006N
EasySort™ Human Naïve CD4 <sup>+</sup> T Cell Isolation Kit	MIH007N
EasySort™ Human Naïve CD8 <sup>+</sup> T Cell Isolation Kit	MIH008N
EasySort™ Mouse Naïve CD4 <sup>+</sup> T Cell Isolation Kit	MIM007N

Product Name	Cat. No.
EasySort™ Mouse Naïve CD8 <sup>+</sup> T Cell Isolation Kit	MIM008N
EasySort™ Mouse CD3 <sup>+</sup> T Cell Isolation Kit	MIM001N
EasySort™ Mouse CD4 <sup>+</sup> T Cell Isolation Kit	MIM002N
EasySort™ Mouse CD8 <sup>+</sup> T Cell Isolation Kit	MIM003N
EasySort™ Mouse B Cell Isolation Kit	MIM004N
EasySort™ Mouse NK Cell Isolation Kit	MIM005N

For more cell isolation products, please visit [www.elabscience.com](http://www.elabscience.com) or contact local distributors.

## T Cell Activation

Activation Method	Principle	Advantage	Disadvantage	Applicable Scenario
Antigen-presenting Cells (APC)	Use APCs such as dendritic cells to present antigen-MHC complexes and express co-stimulatory molecules (e.g., B7)	Physiological activation, suitable for the expansion of specific T cells	Complicated preparation, high cost, large batch-to-batch variation	Research on antigen-specific T cells
Phytohemagglutinin, PMA (PHA/ConA)	Induce polyclonal activation by binding to glycoproteins on the surface of T cells, a non-specific stimulation	Potent activation, low cost	Non-specific activation, high toxicity, unable to induce long-term proliferation	Short-term proliferation experiments, preliminary screening
Soluble Antibodies (CD3/CD28)	Anti-CD3 antibody binds to TCR/CD3, and Anti-CD28 Antibody provides co-stimulatory signals to directly activate T cells	Low cost, flexible operation	Need to optimize the concentration ratio, uneven activation, residual free antibodies may interfere with downstream experiments	Small-scale experiments, pre-research needed
Antibody-coated Magnetic Beads	Immobilize CD3/CD28 antibodies on the surface of magnetic beads, which can provide the main signals and co-stimulatory signals required for T cell activation. The magnetic beads can be removed later	High activation efficiency, stable signals, easy to separate	Slightly higher cost	Short-term rapid expansion, high-purity requirements

### T Cell Activation by CD3/CD28 Magnetic Beads

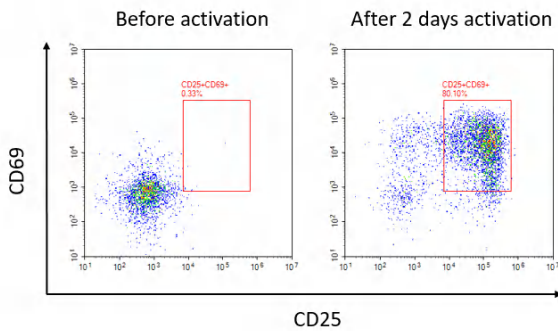


Fig. 6. Human peripheral blood (PBMC) were activated with Human CD3/CD28 T Cell Activation Beads and cultured for 2 days. The expression of CD69 and CD25 before (Left) and after (Right) activation were detected.

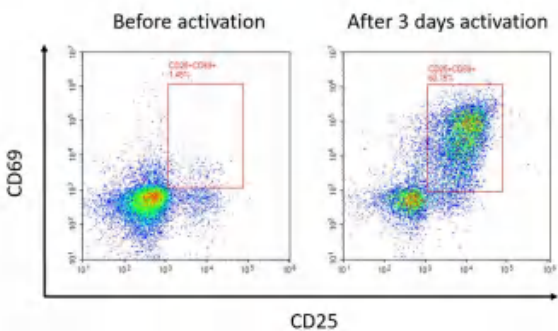


Fig. 8. Mouse splenic T cells were activated with Mouse CD3/CD28 T Cell Activation Beads and cultured for 3 days. The expression of CD69 and CD25 before (Left) and after (Right) activation were detected.

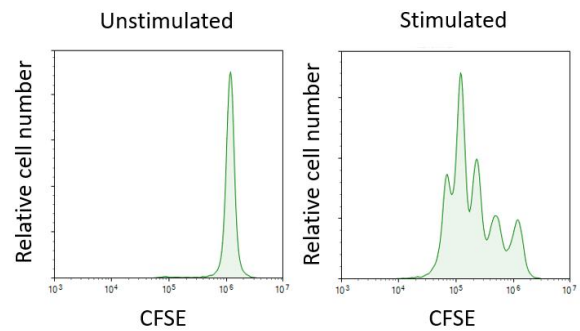


Fig. 7. After sorting CD3<sup>+</sup> T cells from human peripheral blood, the cells were activated with Human CD3/CD28 T Cell Activation Beads and cultured for 3 days. The proliferation of T cells in the non-activated group (Left) and the activated group (Right) were detected by CFSE staining.

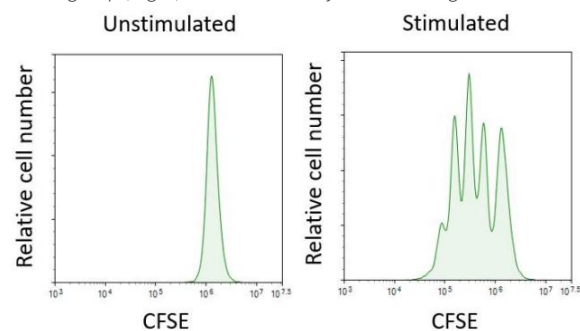


Fig. 9. Mouse splenic T cells were activated with Mouse CD3/CD28 T Cell Activation Beads and cultured for 3 days. The proliferation of T cells in the non-activated group (Left) and the activated group (Right) were detected by CFSE staining.

Product Name	Cat. No.	Application
Human CD3/CD28 T Cell Activation Beads	MIH001A	Human T cell in vitro activation
PE Anti-Human CD25 Antibody[BC96]	E-AB-F1194D	Detection of in vitro activation effect of human T cells
APC Anti-Human CD69 Antibody[FN50]	E-AB-F1138E	
Mouse CD3/CD28 T Cell Activation Beads	MIM001A	Mouse T cell in vitro activation
PE Anti-Mouse CD25 Antibody[PC-61.5.3]	E-AB-F1102D	Detection of in vitro activation effect of mouse T cells
APC Anti-Mouse CD69 Antibody[H1.2F3]	E-AB-F1187E	
CFSE Cell Division Tracker Kit	E-CK-A345	T cell proliferation detection

T Cell Antigen-presenting Cell Activation

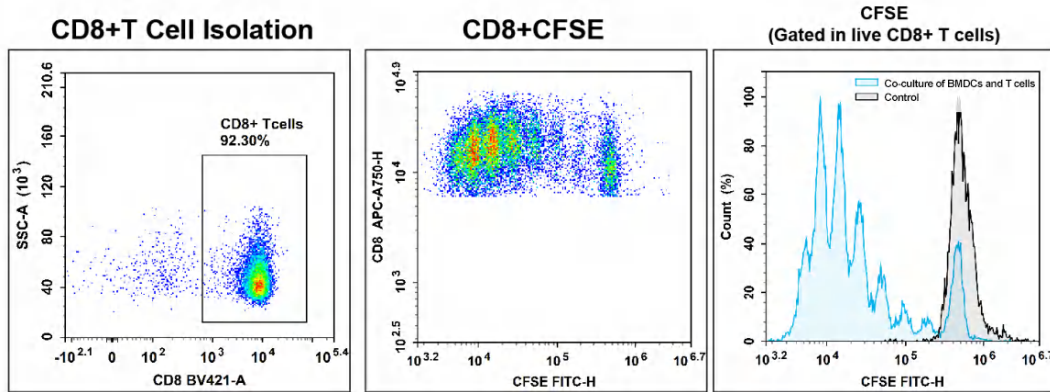


Fig. 10. Flow cytometry was used to detect the purity of CD8<sup>+</sup> T cells sorted from the spleens of C57 mice. The purity of CD8<sup>+</sup> T cells after sorting was 92.30%. Mature dendritic cells (DCs) were loaded with antigens from inactivated target cells (Raw264.7) and then co-cultured with the sorted CD8<sup>+</sup> T cells for 72 h to detect the proliferation of CD8<sup>+</sup> T cells.

Product Name	Cat. No.	Application
Mouse Bone Marrow-derived Dendritic Cells (BMDC) Induction and Identification Kit	XJM003	In vitro culture and maturation promotion of antigen-presenting cells
EasySort™-5 Magnet	EC001	In vitro sorting of mouse splenic T cells
EasySort™ Mouse CD8 <sup>+</sup> T Cell Isolation Kit	MIM003N	
CFSE Cell Division Tracker Kit	E-CK-A345	Detection of T cells proliferation

T Cell PMA Activation

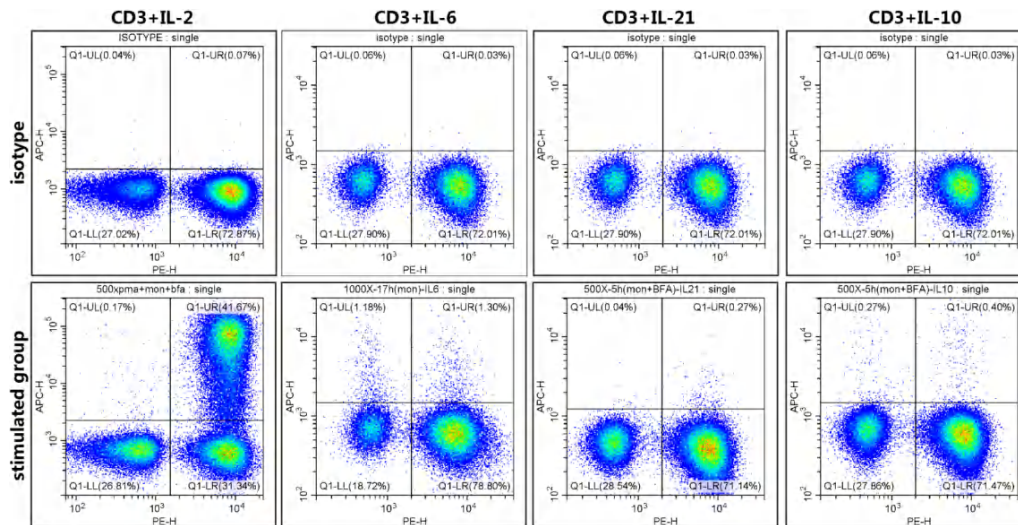


Fig. 11. After human peripheral blood cells (PBMC) were stimulated and blocked with the Cell Stimulation and Protein Transport Inhibitor Kit for 5 h, the expression of cytokines were detected.

Product Name	Cat. No.	Application
Protein Transport Inhibitor MIX	E-CK-A013	BFA and monensin blockers
Cell Stimulation MIX Kit	E-CK-A019	PMA and INM activators
APC Anti-Human IL-2 Antibody[MQ1-17H12]	E-AB-F1200E	Detection of cytokine secretion function
APC Anti-Human IL-6 Antibody[MQ2-13A5]	E-AB-F1206E	
APC Anti-Human IL-10 Antibody[JES3-9D7]	E-AB-F1198E	
APC Anti-Human IL-21 Antibody[3A3-N2]	E-AB-F1202E	
PE Anti-Human CD3 Antibody[UCHT1]	E-AB-F1230D	

# 03 T Cell Detection

In T cell research, commonly used samples include the spleen, lymph nodes, tumors, and peripheral blood. These samples comprise diverse immune cell populations, including T cells, B cells, and NK cells. T cells frequently interact with other immune cells; for example, follicular helper T cells support the differentiation of B cells into plasma cells. Therefore, T cell analysis is often accompanied by simultaneous detection of other immune cell subsets. Elabscience® offers flow cytometry antibodies targeting major immune cell populations across multiple species, providing a robust solution for T cell analysis.

## ■ Human T Cell Detection

### Detection of T/B/NK in Human Peripheral Blood

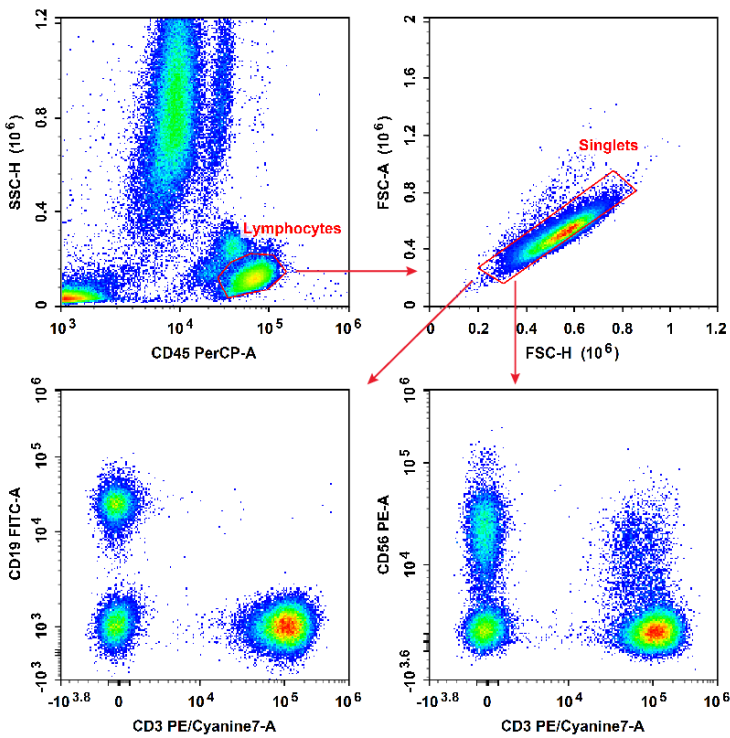


Fig. 12. Detection of T/B/NK in human peripheral blood.

Product Name	Cat. No.
PE/Cyanine7 Anti-Human CD3 Antibody[OKT-3]	E-AB-F1001H
FITC Anti-Human CD19 Antibody[CB19]	E-AB-F1004C
PerCP Anti-Human CD45 Antibody[HI30]	E-AB-F1137F
PE Anti-Human CD56/NCAM Antibody[5.1H11]	E-AB-F1239D
Anti-Human CD19-FITC/CD56-PE/CD3-PE/Cyanine7/CD45-PerCP Cocktail	E-AB-FC0011

- 1.It is recommended to stain human peripheral blood samples with CD45, which is beneficial for the lymphocyte phylum gating through CD45 and SSC.
- 2.CD45<sup>+</sup>CD3<sup>+</sup> cells were T Cells, CD3<sup>+</sup>CD56<sup>+</sup> cells were NKT Cells, CD3<sup>+</sup>CD56<sup>-</sup> cells were NK Cells, CD3<sup>+</sup>CD19<sup>+</sup> cells were B Cells.
- 3.It is recommended to set up single positive tubes to adjust compensation.
- 4.In this panel, it is recommended to set Isotype Control for CD16 and CD56, while other markers can be omitted due to obvious populations.

### Detection of T/B/NKT in Human Peripheral Blood

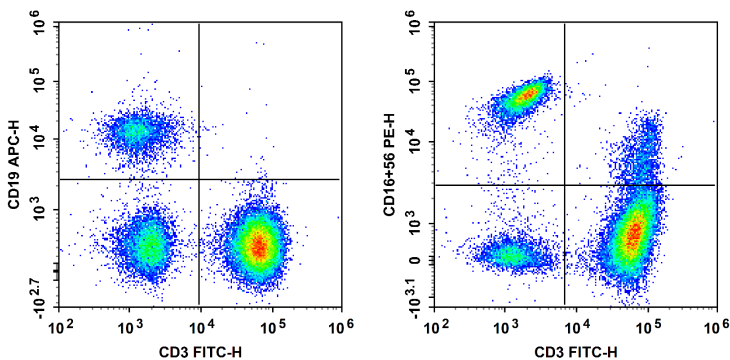


Fig. 13. Detection of T/B/NKT in human peripheral blood.

Product Name	Cat. No.
FITC Anti-Human CD3 Antibody [OKT-3]	E-AB-F1001C
PE Anti-Human CD16 Antibody [3G8]	E-AB-F1236D
APC Anti-Human CD19 Antibody [CB19]	E-AB-F1004E
PE Anti-Human CD56/NCAM Antibody [5.1H11]	E-AB-F1239D
Anti-Human CD3-FITC/CD19-APC/CD16+CD56-PE Cocktail	E-AB-FC0007

- 1.CD3<sup>+</sup> cells were T Cells, CD3<sup>+</sup>CD16<sup>+</sup>/CD56<sup>+</sup> cells were NKT Cells, CD3<sup>+</sup>CD16<sup>-</sup>/CD56<sup>-</sup> cells were NK Cells, CD3<sup>+</sup>CD19<sup>+</sup> cells were B Cells.
- 2.In this panel, it is recommended to set Isotype Control for CD16 and CD56, while other markers can be omitted due to obvious populations.
- 3.It is recommended to set up single positive tubes to adjust compensation.

## Detection of $\gamma/\delta$ T Cells in Human Peripheral Blood

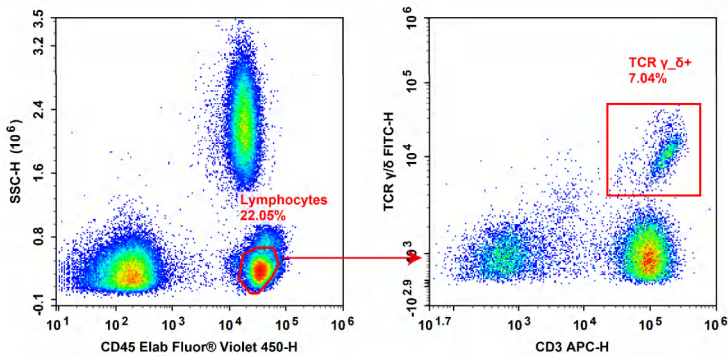


Fig. 14. Detection of  $\gamma/\delta$  T Cells in human peripheral blood.

Product Name	Cat. No.
APC Anti-Human CD3 Antibody[UCHT1]	E-AB-F1230E
Elab Fluor® Violet 450 Anti-Human CD45 Antibody[HI30]	E-AB-F1137Q
FITC Anti-Human TCR $\gamma/\delta$ Antibody[B1]	E-AB-F1145C

1. CD45<sup>+</sup>CD3<sup>+</sup> cells were T Cells, CD45<sup>+</sup>CD3<sup>+</sup>TCR  $\gamma/\delta$ <sup>+</sup> cells were  $\gamma/\delta$  T Cells.
2. In this panel, it is recommended to set Isotype Control for TCR  $\gamma/\delta$ , while other markers can be omitted due to obvious populations.
3. There is no need to set up any single positive tube to adjust compensation.

## Mouse T Cell Detection

### Detection of T/B/NK in Mouse Peripheral Blood

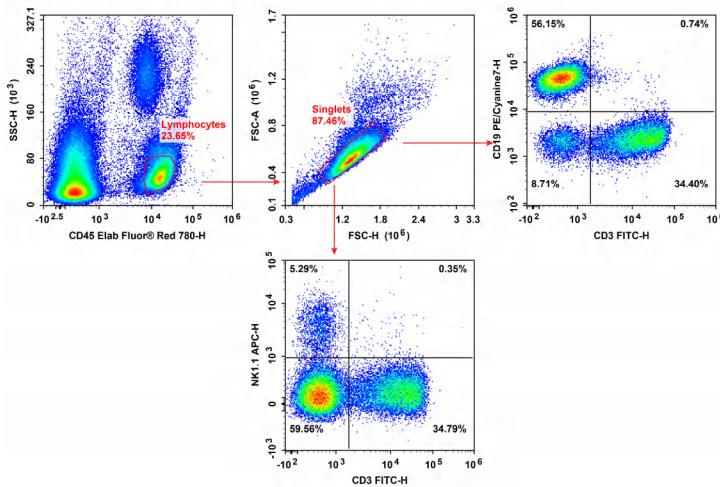


Fig. 15. Detection of T/B/NK in mouse peripheral blood.

Product Name	Cat. No.
FITC Anti-Mouse CD3 Antibody[17A2]	E-AB-F1013C
PE/Cyanine7 Anti-Mouse CD19 Antibody[1D3]	E-AB-F0986H
Elab Fluor® Red 780 Anti-Mouse CD45 Antibody[30-F11]	E-AB-F1136S
APC Anti-Mouse CD161/NK1.1 Antibody[PK136]	E-AB-F0987E

1. Add CD45 to this panel; the lymphocyte populations can be gated directly through CD45 and SSC.
2. The CD3/CD4/CD8 cell populations were obvious, it can effectively distinguish between positive and negative cells even without Isotype Control.
3. The detection markers of NK cells should be selected based on different mouse varieties, typically with C57BL/6 mouse one can use NK1.1, and with BALB/c mouse one can use CD49b (DX5). CD3<sup>+</sup>NK1.1<sup>+</sup> or CD3<sup>+</sup>CD49b<sup>+</sup> cells were NK Cells.
4. The key factor in this experiment is red blood cell lysis. Excessive or insufficient lysis of red blood cells will lead to unclear lymphocyte subpopulations.

### Detection of T/B Cells in Mouse Lymph Node

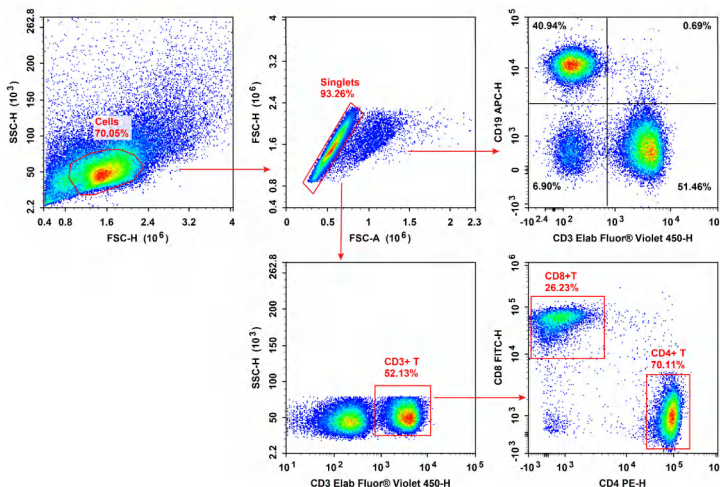


Fig. 16. Detection of T/B cells in mouse lymph node.

Product Name	Cat. No.
Elab Fluor® Violet 450 Anti-Mouse CD3 Antibody[17A2]	E-AB-F1013Q
PE Anti-Mouse CD4 Antibody[GK1.5]	E-AB-F1097D
FITC Anti-Mouse CD8a Antibody[53-6.7]	E-AB-F1104C
APC Anti-Mouse CD19 Antibody[1D3]	E-AB-F0986E

1. The lymph nodes are mainly composed of lymphocytes, so there is no need for CD45 Markers.
2. CD3/CD4/CD8/CD19 cells are easy distinguished, Single Positive, FMO and Isotype Controls are unnecessary.

## Detection of T Cells in Mouse Tumor

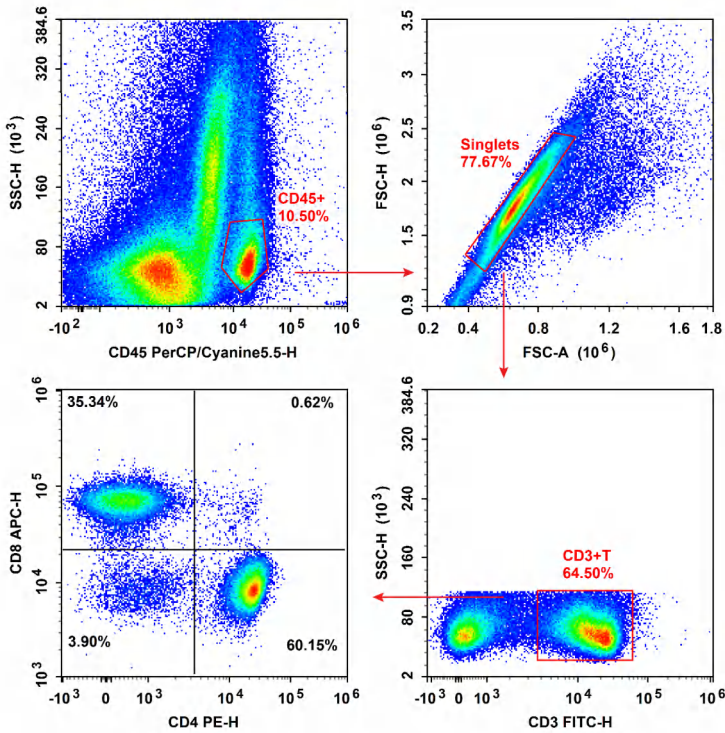


Fig. 17. Detection of T cells in mouse tumor.

Product Name	Cat. No.
FITC Anti-Mouse CD3 Antibody[17A2]	E-AB-F1013C
PE Anti-Mouse CD4 Antibody[GK1.5]	E-AB-F1097D
APC Anti-Mouse CD8a Antibody[53-6.7]	E-AB-F1104E
PerCP/Cyanine5.5 Anti-Mouse CD45 Antibody[30-F11]	E-AB-F1136J

1. Tumor tissues are primarily composed of tumor cells, and the proportion of lymphocytes is relatively low. Lymphocyte populations can be gated by combining CD45 and SSC.
2. The lymphocyte gate in tumor cells were defined as the gate with CD45 high and SSC low.

## ■ Non-human Primate (NHP) T Cell Detection

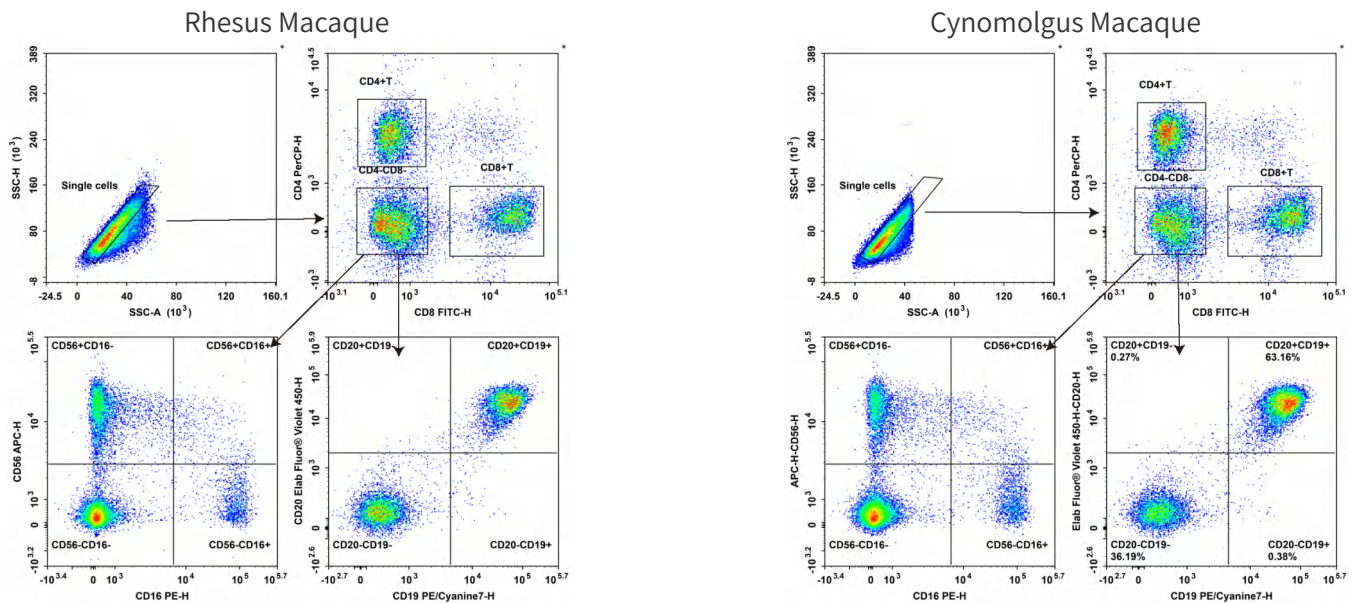


Fig. 18. Detection results of T/B/ NK cells in blood samples of rhesus macaque and cynomolgus macaque.

Product Name	Cat. No.
PerCP Anti-Human CD4 Antibody[SK3]	E-AB-F1352F
FITC Anti-Human CD8 Antibody[SK1]	AN00337C
PE Anti-Human CD16 Antibody[3G8]	E-AB-F1236D
PE/Cyanine7 Anti-Human CD19 Antibody[CB19]	E-AB-F1004H
Elab Fluor® Violet 450 Anti-Human CD20 Antibody[2H7]	E-AB-F1212Q
APC Anti-Human CD56/NCAM Antibody[5.1H11]	E-AB-F1239E

# 04 T Cell Activation Status Detection

In the T cell-mediated immune response, Naïve T cells differentiate into effector T cells and memory T cells after receiving stimuli. The two types of cells are responsible for immune effector function and immune memory respectively. The ratio and quantity of effector T cells to memory T cells are the core markers for evaluating the effectiveness of T cell immune responses. Some cell surface markers that can be used for detecting the activation status of T cells are detailed in the following table:

Base Marker (Backbone Panel)			
Marker	Clone No.	Function	Classification
CD3	UCHT1	The main classification marker of T cells, a protein complex that mediates T cell signaling	Basic Markers for T Cells Detection
CD4	SK3	The main marker of Th cells that plays a role in initiating or enhancing functions in the early stage of T cell activation	
CD8	SK-1	The main marker of cytotoxic T cells, a co-receptor of MHC I molecules, which plays an important role in T cell-mediated killing	
CD45	HI30	Leukocyte markers, regulators of T cell and B cell antigen receptor signaling, regulators of cell growth and differentiation	

Optional Marker (Drop-ins Panel)			
Marker	Clone No.	Function	Classification
CD45RA	HI100	Differentiates between memory T cells and Naïve T cells	Markers for Naïve and Memory T Cells
CD197/CCR7	G043H7	Activates B and T lymphocytes, stimulates the maturation of dendritic cells. When paired with CD45RA, it can distinguish between central memory T cells and effector memory T cells	
CD27	O323	Involved in the generation and long-term maintenance of T cell immunity. Central memory T cells, stem-like memory T cells, and Naïve T cells are positive, while effector memory T cells are negative	Markers for the Early Stage of T Cells Differentiation
CD28	CD28.2	The second messenger for T cell activation, which is related to T cell proliferation, survival, IL-2 production, and Th2 cell development. Central memory T cells, stem-like memory T cells, and Naïve T cells are positive, while effector memory T cells are negative or weakly expressed	
CD62L	DREG56	Involved in the generation and long-term maintenance of T cell immunity. Central memory T cells, stem-like memory T cells, and Naïve T cells are positive, while effector memory T cells are negative	
CD127	A019D5		
CD57	HI57a	Related to the terminal exhaustion state of aging T cells	Markers for the Late Stage of T Cell Differentiation
CD95	DX2	CD95, also known as FAS and APO-1, is a type of death receptor that can induce cell apoptosis. It is positive in activated T cells such as memory and effector T cells and negative in Naïve T cells.	
KLRG1	2F1	A marker of cell senescence or differentiation for CD8 T cell subsets, used to distinguish between short-lived effector (KLRG1 <sup>high</sup> CD127 <sup>low</sup> ) and memory precursor (KLRG1 <sup>low</sup> CD127 <sup>high</sup> ) CD8 T cells	
CD25	CHI621	The $\alpha$ chain of the IL-2 receptor, expressed on regulatory and resting memory T cells, and up-regulated within 24 h after stimulation of the TCR/CD3 complex	Markers for the Activation State of T Cells
CD30	Ki-4	Expressed on activated lymphocytes and can regulate cell growth, proliferation, and apoptosis through multiple signaling pathways	
CD38	HIT2	ADP-ribosyl cyclase, which exerts cell adhesion and signal transduction functions. It is constitutively expressed on Naïve T cells, down-regulated in resting memory cells, and then up-regulated again in activated cells	
CD69	FN50	CD69 is one of the earliest up-regulated markers after T cell activation and is diluted as the cells proliferate	
CD134	Ber-ACT35	A member of the TNF receptor super-family, which can inhibit cell apoptosis	
CD154/CD40L	5C8	Mediates B cell proliferation and IgE production and is involved in immunoglobulin class switching	
HLA-DR	L243	Constitutively expressed on APCs and up-regulated on T cells after stimulation	
CD73	AD2	An extracellular 5'-nucleotidase that can hydrolyze extracellular nucleotides into membrane-permeable nucleotides, highly expressed on the surface of immunosuppressive T cells	Markers for the Exhaustion State of T Cells
CD101	BB27	Inhibits T cell proliferation and is associated with the terminal exhaustion state	
CD152/CTLA-4	BNI3	An inhibitory receptor that inhibits T cell co-stimulatory signals and is a marker of early exhaustion	
CD279/PD-1	EH12.2H7	An inhibitory receptor and the most classic marker of exhaustion. Sustained high-level expression inhibits T cell function	
CD366/Tim-3	RMT3-23 (Mouse)	An immune checkpoint protein that regulates the immune response to prevent over-activation. In the tumor micro-environment, it can inhibit T cell function, leading to immune escape. PD-1 <sup>+</sup> Tim-3 <sup>+</sup> double-positive T cells often represent a highly exhausted T cell subset	
TCR $\gamma/\delta$	B1	A marker for $\gamma/\delta$ T cells	Other T Cells

## Human T Cell Activation State Detection

### Detection of Naïve/Memory T Cells in Human Peripheral Blood

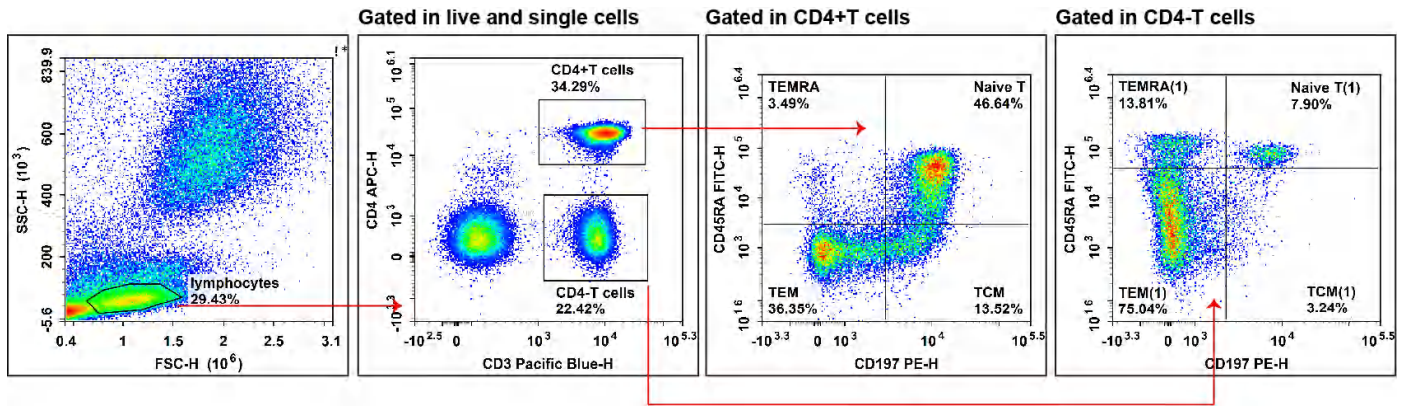


Fig. 19. Detection of Naïve/Memory T cells in human peripheral blood.

Product Name	Cat. No.
Elab Fluor® Violet 450 Anti-Human CD3 Antibody[UCHT1]	E-AB-F1230Q
APC Anti-Human CD4 Antibody[SK3]	E-AB-F1352E
FITC Anti-Human CD45RA Antibody[HI100]	E-AB-F1052C
PE Anti-Human CD197/CCR7 Antibody[G043H7]	E-AB-F1159D

- CD3<sup>+</sup> cells were T cells, CD3<sup>+</sup>CD4<sup>+</sup> were Th cells, Phenotype of Naïve T cells: CD45RA<sup>+</sup>CCR7<sup>-</sup>; Phenotype of effector memory T cells (TEM): CD45RA<sup>+</sup>CCR7<sup>+</sup>; Phenotype of central memory T cells (TCM): CD45RA<sup>+</sup>CCR7<sup>+</sup>.
- It is recommended to set up single positive tubes to adjust compensation.
- In this panel, it is recommended to set Isotype Control for CD45RA and CCR7.

### Detection of CD4 Naïve T Cells in Human Peripheral Blood

#### Gated in live cells

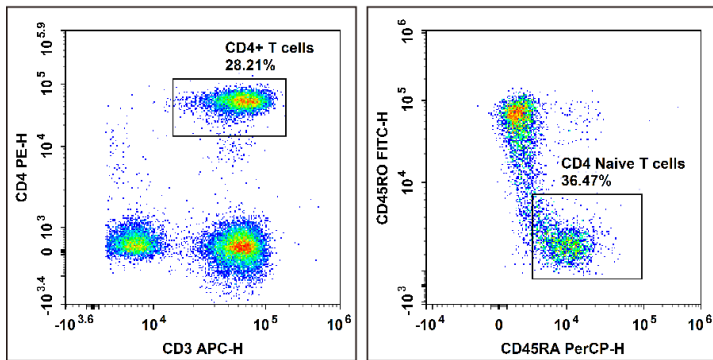


Fig. 20. Detection of CD4 Naïve T cells in human peripheral blood.

Product Name	Cat. No.
APC Anti-Human CD3 Antibody[OKT-3]	E-AB-F1001E
PE Anti-Human CD4 Antibody[SK3]	E-AB-F1352D
PerCP Anti-Human CD45RA Antibody[HI100]	E-AB-F1052F
FITC Anti-Human CD45RO Antibody[UCHL1]	E-AB-F1139C

- Gate the Th cell population by CD3<sup>+</sup>CD4<sup>+</sup>. In the CD4<sup>+</sup> T cell population, identify the CD4<sup>+</sup> Naïve T cells by CD45RA and CD45RO. The phenotype of CD4<sup>+</sup> Naïve T cells was CD45RO<sup>-</sup>CD45RA<sup>+</sup>.
- It is recommended to set up single positive tubes to adjust compensation.
- In this panel, it is recommended to set Isotype Control for CD45RO and CD45RA.

### Detection of CD8 Naïve T Cells in Human Peripheral Blood

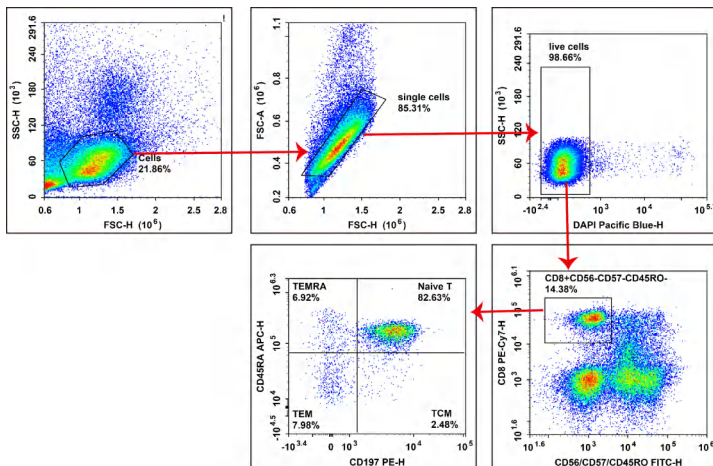


Fig. 21. Detection of CD8 Naïve T cells in human peripheral blood.

Product Name	Cat. No.
PE/Cyanine7 Anti-Human CD8a Antibody[OKT-8]	E-AB-F1110H
APC Anti-Human CD45RA Antibody[HI100]	E-AB-F1052E
FITC Anti-Human CD45RO Antibody[UCHL1]	E-AB-F1139C
FITC Anti-Human CD56/NCAM Antibody[MY31]	E-AB-F1270C
FITC Anti-Human CD57 Antibody[HNK-1]	E-AB-F1067C
PE Anti-Human CD197/CCR7 Antibody[G043H7]	E-AB-F1159D
DAPI Reagent (25µg/mL)	E-CK-A163

- It is recommended to set up single positive tubes to adjust compensation.
- In this panel, it is recommended to set Isotype Control for CD45RO, CD56, CD57, CD8, CD45RA and CD197.
- Exclude most memory T cells, NKT cells, some activated cytotoxic T cells, terminally differentiated effector T cells, and senescent-like T cells by gating on CD45RO<sup>-</sup>CD56<sup>-</sup>CD57<sup>-</sup> cells.
- Phenotype of CD8 Naïve T cells: CD8<sup>+</sup>CD45RA<sup>+</sup>CD197<sup>-</sup>.

Detection of Human T Cell Activation Status

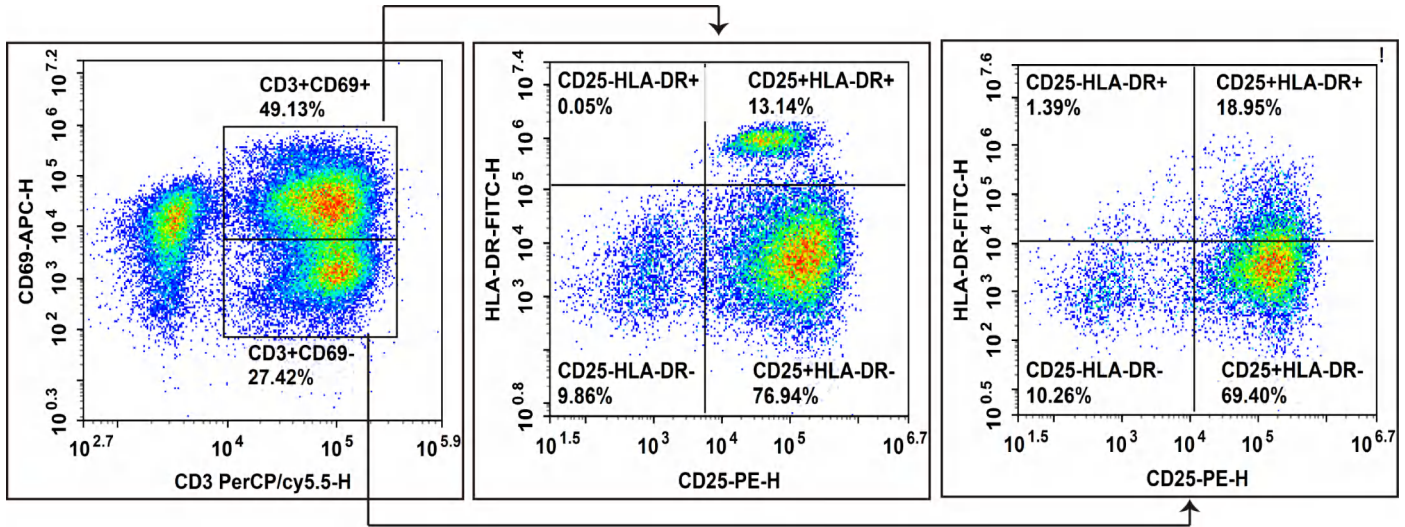


Fig. 22. Human PBMC cells activated with Human CD3/CD28 T Cell Activation Beads and cultured for 48 h, then detect the activation status of T cells.

Product Name	Cat. No.
PerCP/Cyanine5.5 Anti-Human CD3 Antibody[UCHT1]	E-AB-F1230J
PE Anti-Human CD25 Antibody[CHI621]	AN00360D
APC Anti-Human CD69 Antibody[FN50]	E-AB-F1138E
FITC Anti-Human HLA-DR Antibody[L243]	E-AB-F1111C

1. Human peripheral blood (PBMC) cells were divided into CD3<sup>+</sup>CD69<sup>+</sup> and CD3<sup>+</sup>CD69<sup>-</sup> cell populations, based on CD3 and CD69.
2. In the CD3<sup>+</sup>CD69<sup>+</sup> cell population, further analysis was performed using CD25 and HLA-DR. The CD3<sup>+</sup>CD69<sup>+</sup>CD25<sup>+</sup>HLA-DR<sup>-</sup> cell population represents early-activated T cells; the CD3<sup>+</sup>CD69<sup>+</sup>CD25<sup>+</sup>HLA-DR<sup>+</sup> cell population represents activated and proliferating T cells; the CD3<sup>+</sup>CD69<sup>-</sup>CD25<sup>+</sup>HLA-DR<sup>-</sup> cell population represents highly activated T cells, which may be used to assess T cell immune status during acute infection.
3. In the CD3<sup>+</sup>CD69<sup>-</sup> cell population, further analysis was performed using CD25 and HLA-DR. The CD3<sup>+</sup>CD69<sup>-</sup>CD25<sup>+</sup>HLA-DR<sup>+</sup> cell population represents late-stage or continuously-activated T cells, which were used to evaluate the T cell immune status under chronic infection. The CD3<sup>+</sup>CD69<sup>-</sup>CD25<sup>+</sup>HLA-DR<sup>-</sup> cell population represents long-term continuously-activated effector T cells or memory T cells, and the CD3<sup>+</sup>CD69<sup>-</sup>CD25<sup>+</sup>HLA-DR<sup>+</sup> cell population represents T cells in the active proliferation phase.

■ Mouse T Cell Activation State Detection

Detection of Mouse Naïve T/TCM/TEM Cells

Gated in live CD3<sup>+</sup> T cells

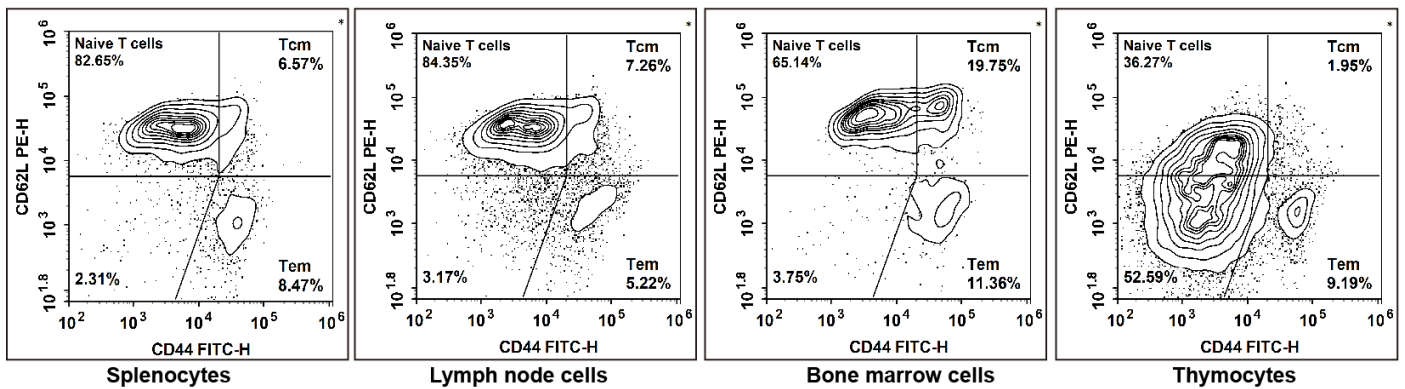


Fig. 23. Detection of mouse Naïve T/TCM/TEM cells.

Product Name	Cat. No.
APC Anti-Mouse CD3 Antibody[17A2]	E-AB-F1013E
FITC Anti-Human/Mouse CD44 Antibody[IM7]	E-AB-F1100C
PerCP/Cyanine5.5 Anti-Mouse CD45 Antibody[30-F11]	E-AB-F1136J
PE Anti-Mouse CD62L Antibody[MEL-14]	E-AB-F1011D

1. T cells were identified by CD45<sup>+</sup>CD3<sup>+</sup>. In CD3<sup>+</sup> T cells, different differentiation stages of T cells were determined by CD44 and CD62L.
2. Phenotype of Naïve T cells: CD62L<sup>+</sup>CD44<sup>-</sup>; Phenotype of TCM cells: CD62L<sup>+</sup>CD44<sup>+</sup>; Phenotype of TEM cells: CD62L<sup>+</sup>CD44<sup>+/low</sup>.
3. It is recommended to set up single positive tubes to adjust compensation.
4. In this panel, it is recommended to set Isotype Control for CD44 and CD62L.

## Detection of Naïve T/TCM/TEM Cells in Mouse Inflammation Model

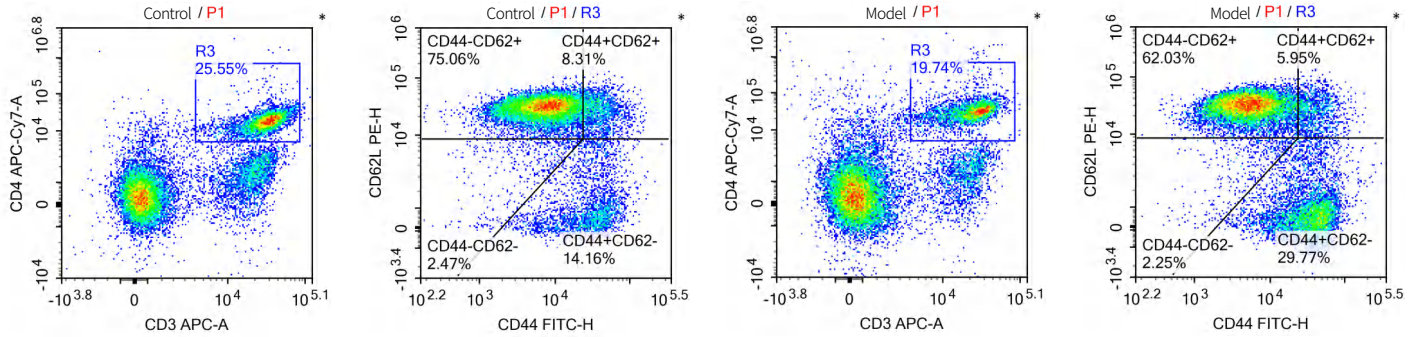


Fig. 24. Detection of Naïve T/TCM/TEM cells in mouse inflammation model.

Product Name	Cat. No.
APC Anti-Mouse CD3 Antibody[17A2]	E-AB-F1013E
Elab Fluor® Red 780 Anti-Mouse CD4 Antibody[GK1.5]	E-AB-F1097S
Elab Fluor® 488 Anti-Human/Mouse CD44 Antibody[IM7]	E-AB-F1100L
PE Anti-Mouse CD62L Antibody[MEL-14]	E-AB-F1011D

The model was a C57 mouse enteritis model: intraperitoneally inject 5 mg/kg of LPS. The control was a normal C57 mice. Spleen cells were collected for flow cytometry detection.

1. Mouse inflammation model: C57 mice, intraperitoneally inject 5 mg/kg of LPS and raise the mice for 7 days.
2. CD4<sup>+</sup> T cells were identified by CD3<sup>+</sup>CD4<sup>+</sup>. Then, different differentiation stages of CD4<sup>+</sup> T cells were determined by CD44 and CD62L.
3. Phenotype of Naïve T cells: CD62L<sup>+</sup>CD44<sup>-</sup>; Phenotype of TCM cells: CD62L<sup>+</sup>CD44<sup>+</sup>; Phenotype of TEM cells: CD62L<sup>-</sup>CD44<sup>+/low</sup>.
4. It is recommended to set up single positive tubes to adjust compensation.
5. In this panel, it is recommended to set Isotype Control for CD44 and CD62L, while other markers can be omitted due to obvious populations.

## Activated T Cell Metabolic Status Detection

As T cells transition from a resting state to an activated state and then differentiate into various subtypes, the metabolic patterns of T cells undergo significant changes to resist pathogens and coordinate the functions of other immune cells. Resting T cells mainly require the ATP-generating process, with oxidative phosphorylation as the main pathway. In contrast, proliferating effector T cells need high metabolic flux through growth-promoting pathways and use glycolysis for rapid energy supply. The metabolic changes of T cells are a key basis for optimizing immunotherapy and are widely used in T cell therapy fields such as CAR-T therapy and tumor immunotherapy. The following table shows some products that can be used for the metabolic detection of activated T cells:

Product Name	Cat. No.	Product Name	Cat. No.
ATP/ADP Ratio Chemiluminescence Assay kit	E-BC-F004	Glycolysis Stress Fluorometric Assay Kit	E-BC-F084
Enhanced ATP Chemiluminescence Assay Kit	E-BC-F201	Glucose (GLU) Fluorometric Assay Kit	E-BC-F037
Enhanced Oxygen Consumption Rate (OCR) Fluorometric Assay Kit	E-BC-F070	Glucose Uptake Fluorometric Assay Kit	E-BC-F041
Extracellular Acidification Rate (ECAR) Fluorometric Assay Kit	E-BC-F069	Glutamine (Gln) Colorimetric Assay Kit	E-BC-K853-M
Fatty Acid Oxidation (FAO) Colorimetric Assay Kit	E-BC-K784-M	Mitochondrial Stress Fluorometric Assay Kit	E-BC-F078
Free Fatty Acids (NEFA/FFA) Fluorometric Assay Kit	E-BC-F039	Pyruvate Fluorometric Assay Kit	E-BC-F058

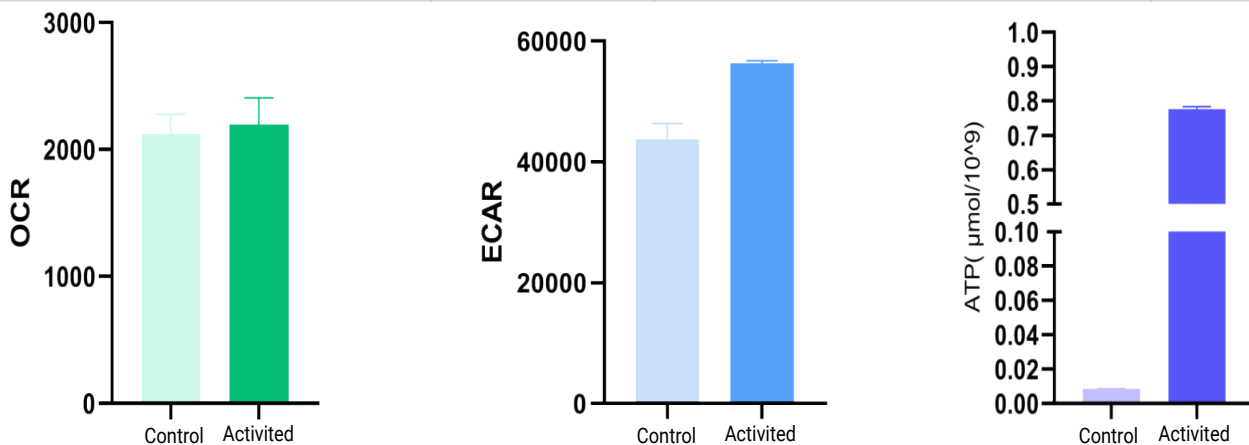


Fig. 25. After activating mouse Naïve CD8 T cells with Mouse CD3/CD28 T Cell Activation Beads for 72 h, detect the metabolic status of the T cells.

# 05 T Cell Cytokine Secretion Ability Assessment

## ■ ELISPOT Detection

ELISPOT (Enzyme-Linked Immunospot Assay) is a highly sensitive technique that can detect and quantitatively analyze the ability of T cells to secrete cytokines (such as IFN- $\gamma$ , IL-2, IL-4, IL-17, etc.) at the single-cell level. This characteristic makes ELISPOT a powerful tool for accurately evaluating the intensity of immune responses and comparing the changes in immune status between different groups or at different time points. It can not only confirm the presence of T cells but also directly and sensitively measure the functional immune responses of T cells.

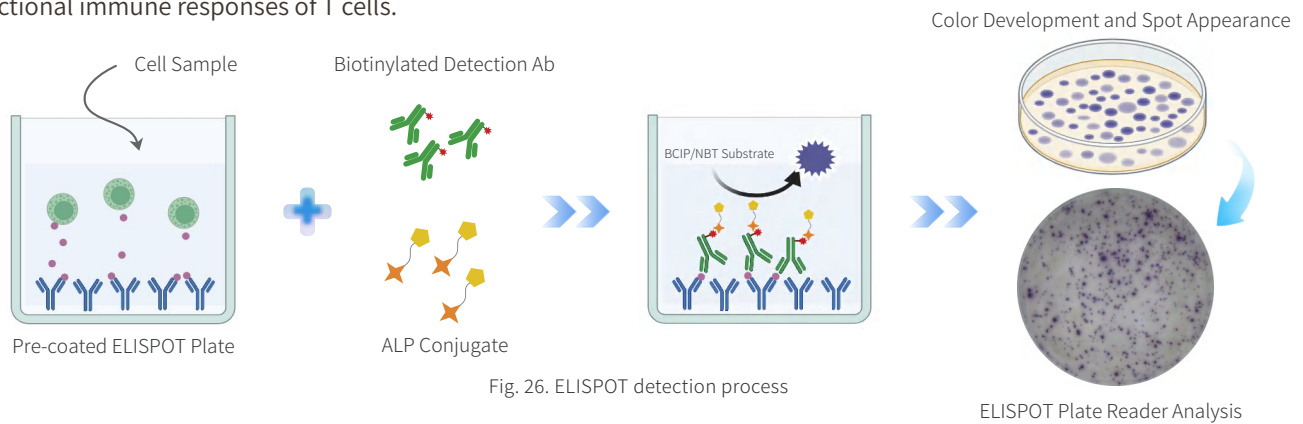


Fig. 26. ELISPOT detection process

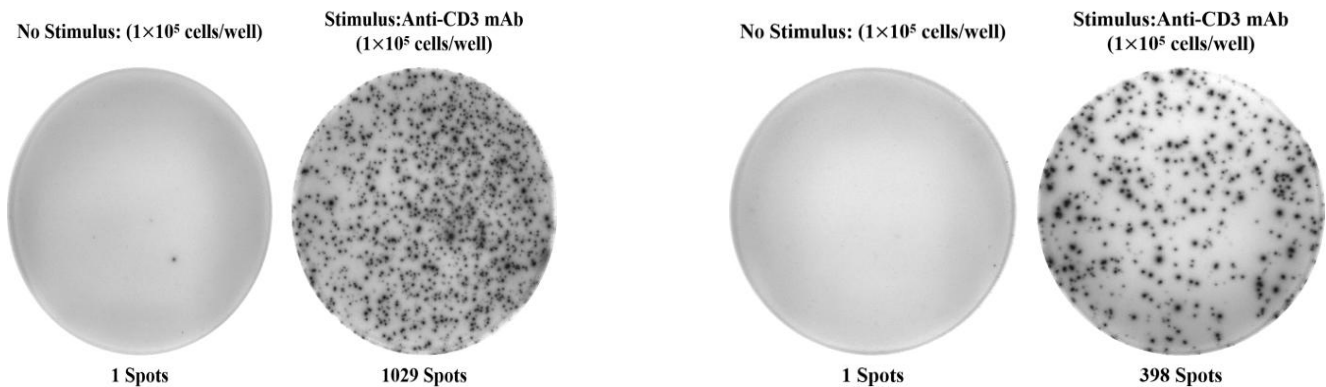


Fig. 27. Incubate human PBMCs (1 × 10<sup>5</sup> cells/well) for 20 h in the absence or presence of the stimulant Anti-CD3 mAb (1:500). The number of spots represents the quantity of cells secreting IFN- $\gamma$ .

Fig. 28. Incubate human PBMCs (1 × 10<sup>5</sup> cells/well) for 20 h in the absence or presence of the stimulant Anti-CD3 mAb (1:500). The number of spots represents the quantity of cells secreting IL-17A.

Product Name	Cat. No.
Human IFN- $\gamma$ (Interferon Gamma) ELISPOT Kit	ESP-H0002
Human IL-1 $\beta$ (Interleukin 1 $\beta$ ) ELISPOT Kit	ESP-H0005
Human IL-2 (Interleukin 2) ELISPOT Kit	ESP-H0006
Human IL-4 (Interleukin 4) ELISPOT Kit	ESP-H0007
Human IL-5 (Interleukin 5) ELISPOT Kit	ESP-H0008
Human IL-6 (Interleukin 6) ELISPOT Kit	ESP-H0009
Human IL-10 (Interleukin 10) ELISPOT Kit	ESP-H0003
Human IL-17A (Interleukin 17A) ELISPOT Kit	ESP-H0004
Human TNF- $\alpha$ (Tumor Necrosis Factor Alpha) ELISPOT Kit	ESP-H0010
Mouse IFN- $\gamma$ (Interferon Gamma) ELISPOT Kit	ESP-M0001

## ■ ELISA Detection of Cytokines in Cell Supernatants

Elabscience® offers high-quality ELISA kits for the quantitative detection of cytokines in humans, mice, and other species. The recognized targets include IFN- $\gamma$ , IL-2, IL-6, IL-1 $\beta$ , etc. To address the industry challenge of poor detection results for cytokines in cell culture supernatants, Elabscience® has developed a series of CellaQuant™ kits, which assist in the detection of the cytokine secretion capacity of cultured T cells.

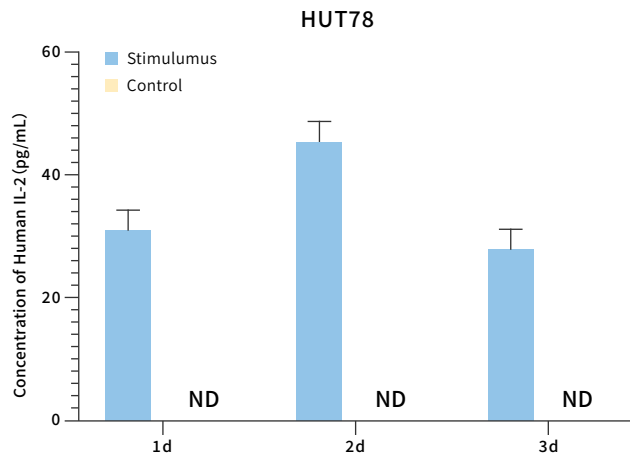


Fig. 29. HUT78 cells ( $5 \times 10^5$  cells/mL) were either treated or untreated with 50 ng/mL TPA and 10  $\mu$ g/mL LPS and cultured for 1, 2 and 4 days. The IL-2 content in the cell culture supernatants were determined by ELISA (ND=Not detected).

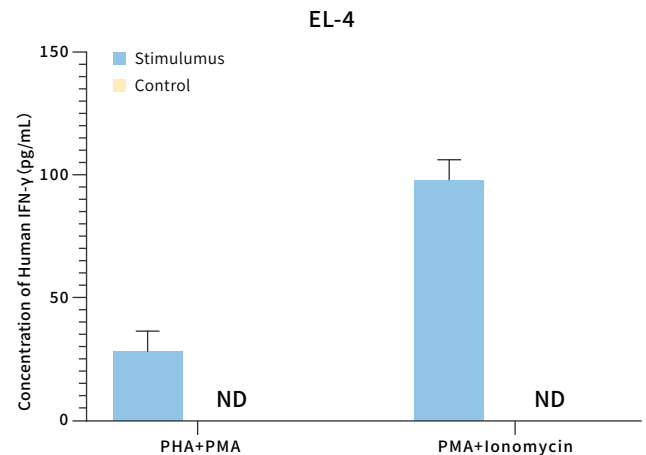


Fig. 30. EL-4 cells ( $1 \times 10^5$  cells/mL) were either treated or untreated with 10  $\mu$ g/mL PHA and 10 ng/mL PMA, or with PMA (50 ng/mL) and Ionomycin (500 ng/mL), and cultured for 1 day. The IFN- $\gamma$  content in the cell culture supernatants were determined by ELISA (ND=Not detected).

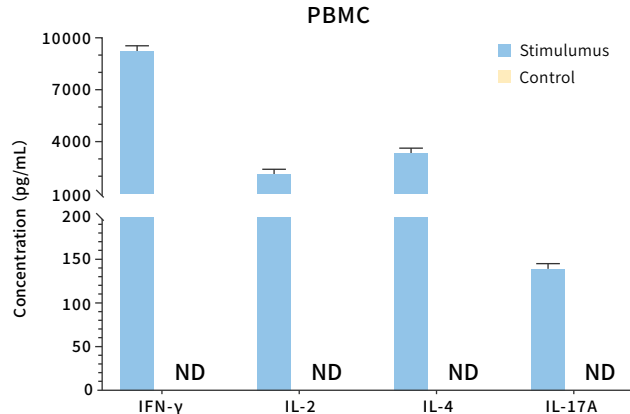


Fig. 31. PBMC cells ( $1 \times 10^5$  cells/mL) were either treated or untreated with 10  $\mu$ g/mL Anti-CD3 and 1  $\mu$ g/mL Anti-CD28 monoclonal antibodies and cultured for 1 day. The contents of Human IFN- $\gamma$ , IL-2, IL-4 and IL-17A in the cell culture supernatants were determined by ELISA (ND=Not detected).

Product Name	Cat. No.
CellaQuant™ Human IFN- $\gamma$ (Interferon Gamma) ELISA Kit	CQH003
CellaQuant™ Human IL-1 $\alpha$ (Interleukin 1 Alpha) ELISA Kit	CQH005
CellaQuant™ Human IL-6 (Interleukin 6) ELISA Kit	CQH001
CellaQuant™ Human IL-8 (Interleukin 8) ELISA Kit	CQH004
CellaQuant™ Human IL-10 (Interleukin 10) ELISA Kit	CQH002
CellaQuant™ Human KIM-1 (Kidney Injury Molecule 1) ELISA Kit	CQH006
CellaQuant™ Mouse IFN- $\gamma$ (Interferon Gamma) ELISA Kit	CQM005
CellaQuant™ Mouse IL-2 (Interleukin 2) ELISA Kit	CQM006
CellaQuant™ Mouse IL-6 (Interleukin 6) ELISA Kit	CQM001
CellaQuant™ Mouse IL-10 (Interleukin 10) ELISA Kit	CQM004
CellaQuant™ Mouse TNF- $\alpha$ (Tumor Necrosis Factor Alpha) ELISA Kit	CQM002
CellaQuant™ Mouse VEGF-A (Vascular Endothelial Cell Growth Factor A) ELISA Kit	CQM007

# 06 Effector T Cell Detection

Effector T cells are the primary executors of the immune response. They mainly originate from naïve T cells following activation, proliferation, and differentiation driven by antigen stimulation and co-stimulatory signals. When T cells are stimulated by antigen-presenting cells (APCs), they recognize antigen peptide-MHC complexes via the T cell receptor (TCR) together with co-stimulatory molecule signals. Subsequently, under the regulation of various cytokine environments, they differentiate into distinct effector T cell subsets with diverse functions. These cells can rapidly migrate to sites of infection or inflammation, where they eliminate pathogens, kill target cells, and regulate immune responses.

Effector T cells mainly include CD4<sup>+</sup> effector T cells (helper T cells) and CD8<sup>+</sup> effector T cells (cytotoxic T cells). CD4<sup>+</sup> effector T cells further differentiate into multiple subsets under the influence of the cytokine microenvironment, such as Th1, Th2, Th17, and Tfh cells. Th1 cells enhance macrophage activity and promote cell-mediated immunity by secreting cytokines such as IFN- $\gamma$ . Th2 cells mainly secrete IL-4, IL-5, and IL-13, regulating humoral immunity and allergic responses. Th17 cells, with IL-17 as their main effector molecule, participate in inflammatory responses and mucosal immunity. Tfh cells play a critical role in germinal center reactions by promoting B cell differentiation and antibody production. CD8<sup>+</sup> effector T cells, represented by cytotoxic T lymphocytes (CTLs), can directly kill virus-infected cells or tumor cells through the secretion of perforin and granzymes or via the Fas-FasL pathway, making them key effector cells in antiviral and antitumor immunity.

## ■ Helper T Cell Detection

Helper T cells are differentiated from Naïve CD4<sup>+</sup> T cells after being stimulated by specific factors (antigens or cytokines). Based on the different cytokines secreted by activated CD4<sup>+</sup> T cells, CD4<sup>+</sup> T cells are classified as either Th1, Th2, Th17, regulatory T (Treg), Th9, Th22, or Tfh cells. Each subset is activated by a specific set of cytokines and transcription factors and are characterized by the cytokines they secrete and the effector functions they perform. Th cells provide auxiliary functions for other cells in the immune system, particularly antigen-presenting cells (APCs) such as macrophages, dendritic cells, and B cells. Therefore, they play an important role in the activation and maturation of these cells. The characteristic markers of each helper T cell subset are listed in the following table:

Cell Classification	Surface Marker	Secreted Cytokine	Cytokine and Chemokine Receptor	Transcription Factor
Th1	CD4, CD183(CXCR3)	IL-1b, IL-2, IL-12, TNF- $\alpha$ , IFN- $\gamma$	CD183(CXCR3), CD195(CCR5), CD197(CCR7), CXCL9, CXCL10, CXCL11	T-bet, STAT4, STAT1
Th2	CD4, CD194(CCR4), CD294(CRTH2)	IL-4, IL-5, IL-10, TGF- $\beta$	CD193(CCR3), CD194(CCR4), CD198(CCR8), MDC, TCA3, TARC	GATA3, IRF4
Th9	CD4, CD196(CCR6)	IL-9, IL-3, IL-21	CCL20, CD196(CCR6)	IRF4, PU.1
Th17	CD4, CD161, CD194(CCR4), CD196(CCR6), IL23R	IL-17A, IL-17F, IL-21, IL-22, IL-23	CCL4, CCL17, CCL22	IRF4, ROR $\gamma$ T
Th22	CD4, CCR10, CD194(CCR4), CD196(CCR6)	IL-22, TNF- $\alpha$ , IL-13	CD194(CCR4), CD196(CCR6), CCR10, PD-1	FOXO4, AHR
Tfh	CD4, CD185 (CXCR5)	IL-21, IL-4	CD185 (CXCR5), CD196(CCR6)	Bcl-6, STAT3, c-Maf
Treg	CD25, CD127, CD152(CTLA4)	IL-4, IL-10, TGF- $\beta$	CD185 (CXCR5), CD196(CCR6)	Foxp3

## Detection of Human Helper T Cells

### Detection of Th1/Th2 Cells in Human Peripheral Blood

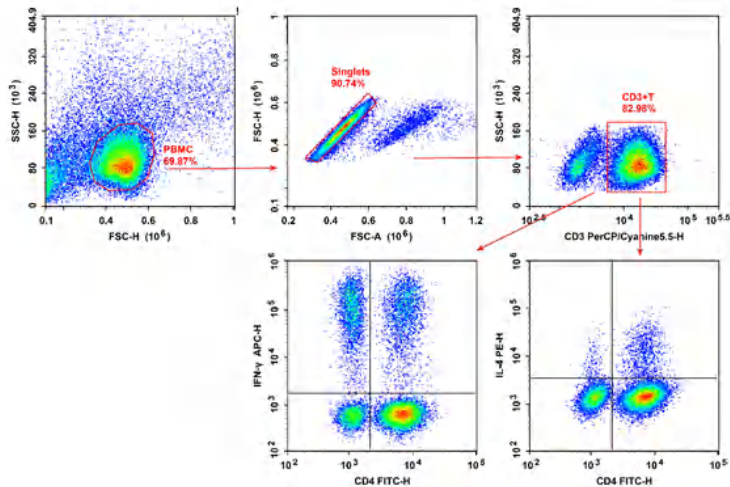


Fig. 32. Detection of Th1/Th2 cells in human peripheral blood.

1. After PBMC sorting, cells must initially be stimulated and blocked with a cytokine stimulation blocker for culture (in this experiment, the reagent used was the Cell Stimulation and Protein Transport Inhibitor Kit (E-CK-A091); the culture condition was 1 h of stimulation and blocking for 4.5 h). Subsequently, one must collect the cells for future flow cytometry experiments. (You can also directly use the Elabscience® Human Th1/Th2 Flow Cytometry Staining Kit (XJH001), which contains flow cytometry antibodies, stimulation blockers, and fixation and permeabilization solutions required for the experiment).
2. PMA stimulation can cause partial endocytosis of CD4 on the surface of human T cells. Therefore, one should use CD4 with clone number SK3, which has less impact on endocytosis.
3. Isotype controls are required for IFN- $\gamma$  and IL-4 because the expression levels of cytokines are generally low.
4. Phenotype of Th1 cells: CD3<sup>+</sup>CD4<sup>+</sup>IFN- $\gamma$ <sup>+</sup>, phenotype of Th2 cells: CD3<sup>+</sup>CD4<sup>+</sup>IL-4<sup>+</sup>.
5. The permeabilization agent causes relatively large damage to cells. It is recommended to disperse the cell pellet formed after centrifugation into a cell suspension before adding the permeabilization agent to reduce cell damage.

Product Name	Cat. No.
Human PBMC Separation Solution (P 1.077)	E-CK-A103
Cell Stimulation and Protein Transport Inhibitor Kit	E-CK-A091
Intracellular Fixation/Permeabilization Buffer Kit	E-CK-A109
PerCP/Cyanine5.5 Anti-Human CD3 Antibody[OKT-3]	E-AB-F1001J
Elab Fluor® 488 Anti-Human CD4 Antibody[SK3]	E-AB-F1352L
APC Anti-Human IFN- $\gamma$ Antibody[B27]	E-AB-F1196E
PE Anti-Human IL-4 Antibody[MP4-25D2]	E-AB-F1203D
Human Th1/Th2 Flow Cytometry Staining Kit	XJH001

### Detection of Th17 Cells in Human Peripheral Blood

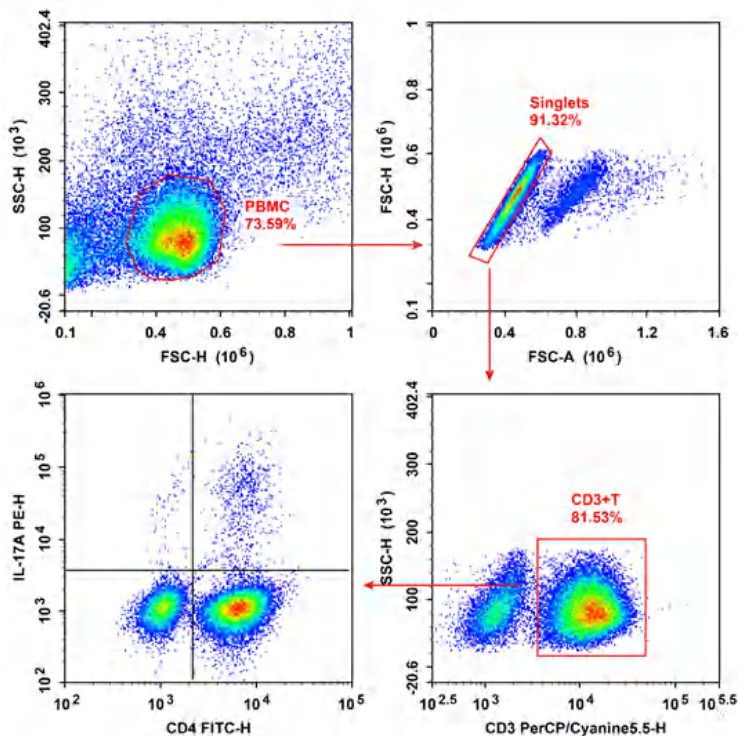


Fig. 33. Detection of Th17 cells in human peripheral blood.

Product Name	Cat. No.
Human PBMC Separation Solution (P 1.077)	E-CK-A103
Cell Stimulation and Protein Transport Inhibitor Kit	E-CK-A091
Intracellular Fixation/Permeabilization Buffer Kit	E-CK-A109
PerCP/Cyanine5.5 Anti-Human CD3 Antibody[OKT-3]	E-AB-F1001J
Elab Fluor® 488 Anti-Human CD4 Antibody[SK3]	E-AB-F1352L
PE Anti-Human IL-17A Antibody[BL168]	E-AB-F1173D

1. After PBMC sorting, cells must first be stimulated and blocked with a cytokine stimulation blocker for culture (in the sample experiment, the reagent used was the Cell Stimulation and Protein Transport Inhibitor Kit (E-CK-A091); the culture condition was 1 h of stimulation and blocking for 4.5 h). Subsequently, one must collect the cells for later flow cytometry experiments. (One can also directly use the Elabscience® Human Th17 Flow Cytometry Staining Kit (XJH002), which contains flow cytometry antibodies, stimulation blockers, and fixation and permeabilization solutions required for the experiment).
2. PMA stimulation can cause partial endocytosis of CD4 on the surface of human T cells. Therefore, one should use CD4 with clone number SK3, which has less impact on endocytosis.
3. Isotype control is required for IL-17A because the expression levels of IL-17A is generally low.
4. Phenotype of Th17 cells: CD3<sup>+</sup>CD4<sup>+</sup>IL-17A<sup>+</sup>.
5. The permeabilization agent causes relatively extensive damage to cells. Therefore, it is recommended to disperse the cell pellet formed after centrifugation into a cell suspension, before adding the permeabilization agent, to reduce cell damage.

## Mouse Helper T Cell Detection

### Detection of Th1/Th2 Cells in Mouse Spleen

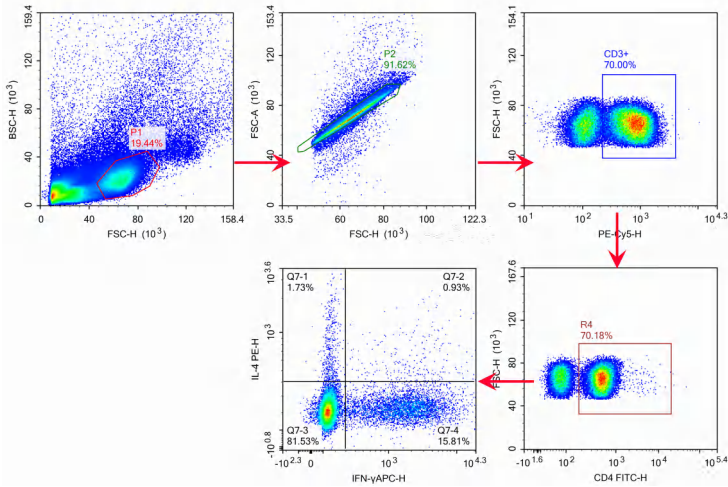


Fig. 34. Detection of Th1/Th2 cells in mouse spleen.

1. After the preparation and counting of the single-cell suspension from the spleen are completed, cells must initially be stimulated and blocked with a cytokine stimulation blocker for culture (in this experiment, the reagent used was the Cell Stimulation and Protein Transport Inhibitor Kit (E-CK-A091); the culture condition was 1 h of stimulation and blocking for 4.5 h). After stimulation and blocking, one must collect the cells for subsequent flow cytometry experiments. (One can also directly use the Elabscience® Mouse Th1/Th2 Flow Cytometry Staining Kit (XJM001), which contains flow cytometry antibodies, stimulation blockers, and fixation and permeabilization solutions required for the experiment).
2. PMA stimulation can cause partial endocytosis of CD4 on the surface of mouse T cells. Therefore, we should use CD4 with clone number RM4-5, which has less impact on endocytosis.
3. Isotype controls are required for IFN- $\gamma$  and IL-4 because the expression levels of cytokines are generally low.
4. Phenotype of Th1 cells: CD3<sup>+</sup>CD4<sup>+</sup>IFN- $\gamma$ <sup>+</sup>, phenotype of Th2 cells: CD3<sup>+</sup>CD4<sup>+</sup>IL-4<sup>+</sup>.
5. The permeabilization agent causes relatively large damage to cells. It is recommended to disperse the cell pellet formed after centrifugation into a cell suspension before adding the permeabilization agent to reduce cell damage.

Product Name	Cat. No.
Cell Stimulation and Protein Transport Inhibitor Kit	E-CK-A091
Intracellular Fixation/Permeabilization Buffer Kit	E-CK-A109
PE/Cyanine5 Anti-Mouse CD3 Antibody[17A2]	E-AB-F1013G
FITC Anti-Mouse CD4 Antibody[RM4-5]	E-AB-F1353C
APC Anti-Mouse IFN- $\gamma$ Antibody[XMG1.2]	E-AB-F1101E
PE Anti-Mouse IL-4 Antibody[11B11]	E-AB-F1204D
Mouse Th1/Th2 Flow Cytometry Staining Kit	XJM001

### Detection of Th17 Cells in Mouse Spleen

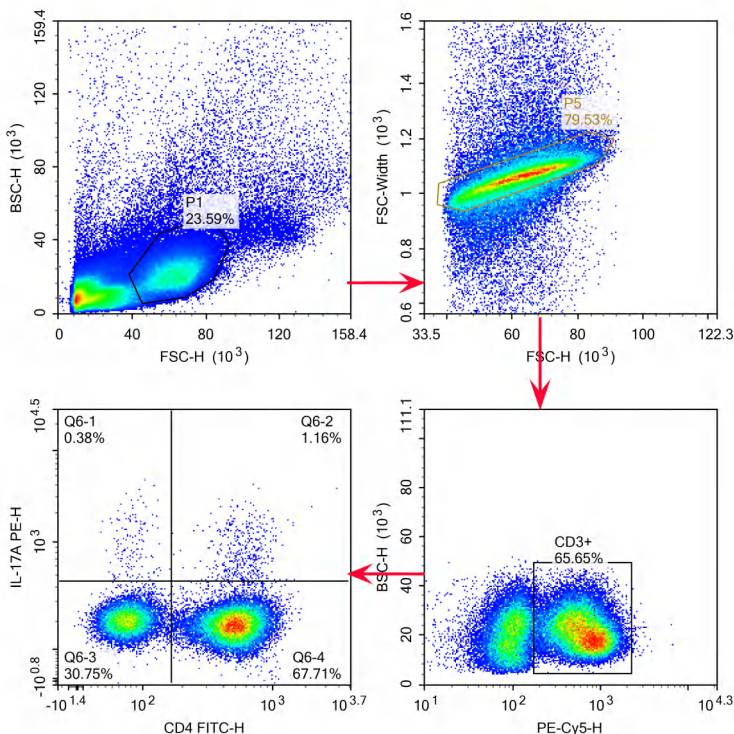


Fig. 35. Detection of Th17 cells in mouse spleen.

Product Name	Cat. No.
Cell Stimulation and Protein Transport Inhibitor Kit	E-CK-A091
Intracellular Fixation/Permeabilization Buffer Kit	E-CK-A109
PE/Cyanine5 Anti-Mouse CD3 Antibody[17A2]	E-AB-F1013G
FITC Anti-Mouse CD4 Antibody[RM4-5]	E-AB-F1353C
PE Anti-Mouse IL-17A Antibody[17F3]	E-AB-F1272D
Mouse Th17 Flow Cytometry Staining Kit	XJM002

1. After the preparation and counting of the single-cell suspension from the spleen are completed, cells must first be stimulated and blocked with a cytokine stimulation blocker for culture (in this experiment, the reagent used was the Cell Stimulation and Protein Transport Inhibitor Kit (E-CK-A091); the culture condition was 1 h of stimulation and blocking for 4.5 h). Then, collect the cells for subsequent flow cytometry experiments. (One can also directly use the Elabscience® Mouse Th17 Flow Cytometry Staining Kit (XJM002), which contains flow cytometry antibodies, stimulation blockers, and fixation and permeabilization solutions required for the experiment).
2. PMA stimulation can cause partial endocytosis of CD4 on the surface of mouse T cells. Therefore, we should use CD4 with clone number RM4-5, which has less impact on endocytosis.
3. Isotype control is required for IL-17A because the expression levels of IL-17A is generally low.
4. Phenotype of Th17 cells: CD3<sup>+</sup>CD4<sup>+</sup>IL-17A<sup>+</sup>.
5. The permeabilization agent causes relatively large damage to cells. Therefore, it is recommended to disperse the cell pellet formed after centrifugation into a cell suspension, before adding the permeabilization agent to reduce cell damage.

## ■ Cytotoxic T Cell Detection

Cytotoxic T cells (CD8<sup>+</sup>T cells) play an important role in immune response processes such as host defense against viral infections and elimination of tumor cells. During the immune response, naïve CD8<sup>+</sup> T cells can specifically recognize the MHC I-antigen peptide complexes on the surface of antigen-presenting cells (such as DC cells) and get activated. Then they differentiate into effector CD8<sup>+</sup> T cells. Effector CD8<sup>+</sup> T cells have strong cytotoxicity and secrete a variety of cytokines. Based on the specific cytokines they secrete, the strength of their cytotoxicity, and their different functions, effector CD8<sup>+</sup>T cells can be further divided into different effector subtypes. Among them, the three main effector subtypes are Tc1 cells (Type 1 Cytotoxic T Cells), Tc2 cells (Type 2 Cytotoxic T Cells), and Tc17 cells (Type 17 Cytotoxic T Cells). The characteristic markers of each cytotoxic T cell subset are listed in the following table:

Cell Classification	Surface Marker	Differentiation Inducers	Secreted Cytokine	Transcription Factor
Tc1	CD49d	IL-2, IL-12	Perforin, granzyme B, IFN- $\gamma$ , TNF- $\alpha$	STAT4, T-bet, EOMES
Tc2	CysIT1, BLT-1	IL-4	IL-4, IL-5, IL-13	STAT6, GATA3
Tc9	IL-9R	IL-4, TGF- $\beta$	IL-9, IFN- $\gamma$	STAT6, IRF4
TC17	CD161, CD26, CD6, CD39, CD69, CD120b, PD-1	IL-6, TGF- $\beta$ , IL-1 $\beta$ , IL-21, IL-23	IL-17, IL-22, GM-CSF	STAT3, ROR $\gamma$ t
TC22	CD122, Ly49	IL-6, IL-21, TNF- $\alpha$	IL-22, IL-17	AhR, STAT1, STAT3, STAT5
Tfcs	CXCR5	IL-6, IL-21, IL-23, TGF- $\beta$	IL-4, IL-21, IFN- $\gamma$	TCF-1, BCL-6, E2a, Runx3
Qa1-restricted CD8 <sup>+</sup> Tregs	CD122, Ly49	IL-15	TGF- $\beta$ , perforin	Eomes
Foxp3 <sup>+</sup> CD8 <sup>+</sup> Tregs	CD103	TGF- $\beta$	IL-10, TGF- $\beta$	Foxp3

## Human Cytotoxic T Cell Detection

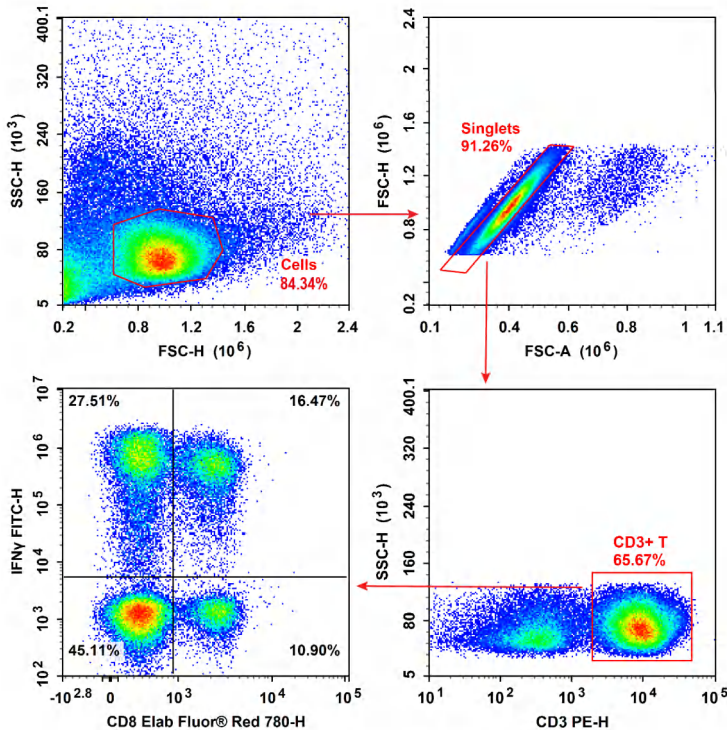


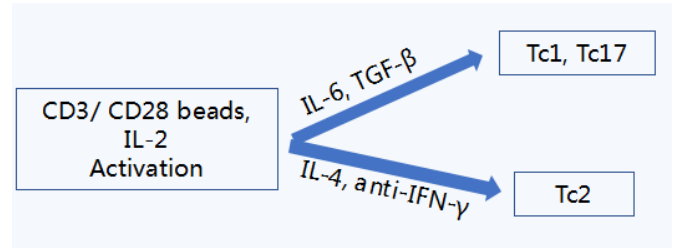
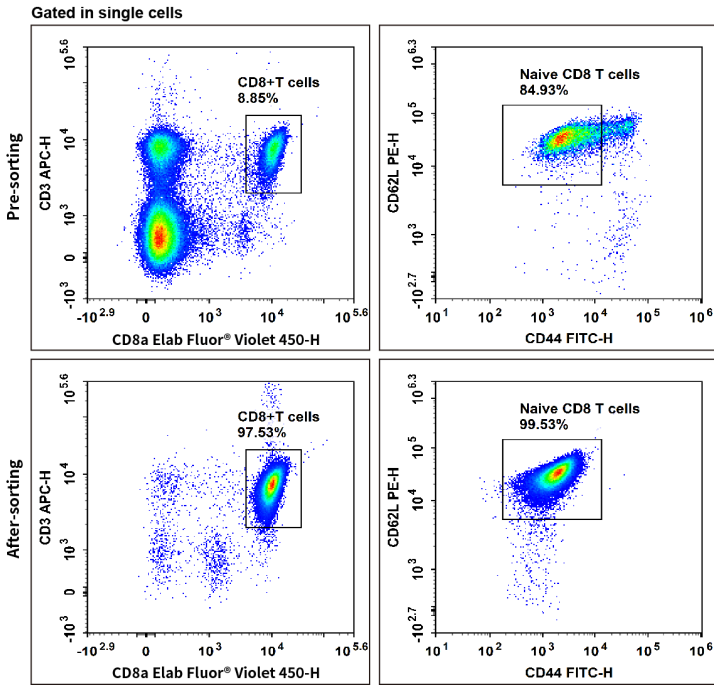
Fig. 36. Detection of human peripheral blood Tc1 cells.

Product Name	Cat. No.
Human PBMC Separation Solution (P 1.077)	E-CK-A103
Cell Stimulation and Protein Transport Inhibitor Kit	E-CK-A091
Intracellular Fixation/Permeabilization Buffer Kit	E-CK-A109
PE Anti-Human CD3 Antibody[UCHT1]	E-AB-F1230D
Elab Fluor® Red 780 Anti-Human CD8a Antibody[OKT-8]	E-AB-F1110S
FITC Anti-Human IFN- $\gamma$ Antibody[B27]	E-AB-F1196C

1. After PBMC sorting, cells need to be stimulated and blocked with a cytokine stimulation blocker for culture first (in this experiment, the reagent used was the Cell Stimulation and Protein Transport Inhibitor Kit (E-CK-A091); the culture condition was 1 h of stimulation and blocking for 4.5 h). Then collect the cells for subsequent flow cytometry experiments.
2. Isotype control is required for IFN- $\gamma$  because the expression levels of IFN- $\gamma$  is generally low.
3. Phenotype of Tc1 cells: CD3<sup>+</sup>CD8<sup>+</sup>IFN- $\gamma$ <sup>+</sup>.
4. The permeabilization agent causes relatively large damage to cells. It is recommended to disperse the cell pellet formed after centrifugation into a cell suspension before adding the permeabilization agent to reduce cell damage.

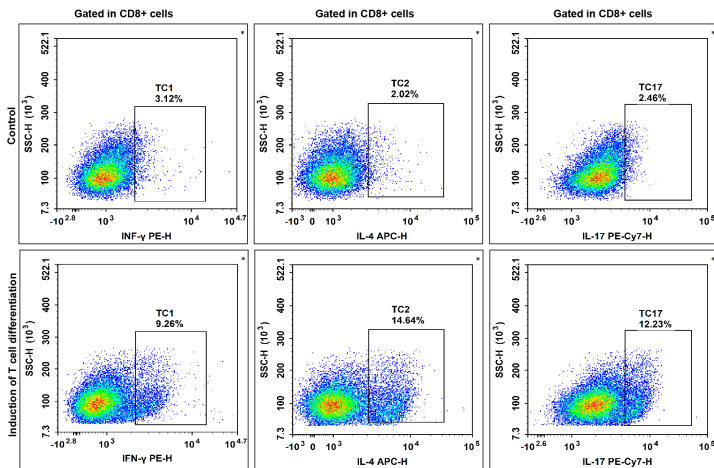
Product Name	Cat. No.	Application
Human IFN- $\gamma$ (Interferon Gamma) ELISPOT Kit	ESP-H0002	Detection of Cytokine Secretion Function of CD8 <sup>+</sup> T Cells
Human IL-4 (Interleukin 4) ELISPOT Kit	ESP-H0007	
Human IL-10 (Interleukin 10) ELISPOT Kit	ESP-H0003	
Human IL-17A (Interleukin 17A) ELISPOT Kit	ESP-H0004	
Human TNF- $\alpha$ (Tumor Necrosis Factor Alpha) ELISPOT Kit	ESP-H0010	Detection of Killing Function of CD8 <sup>+</sup> T Cells
Human GzmB (Granzyme B) ELISA Kit	E-EL-H1617	
Human PRF1(Perforin 1) ELISA Kit	E-EL-H1123	
Mini Sample Human GzmB (Granzyme B) ELISA Kit	E-MSEL-H0019	

Mouse Cytotoxic T Cell Detection



1. After high-purity CD8 Naïve T cells were sorted from the splenocytes of C57 mice using a sorting kit, the purity of CD8 Naïve T cells before and after sorting was detected using APC-CD3/Elab Fluor® Violet 450-CD8a/FITC-CD44/PE-CD62 Antibodies. In the CD3<sup>+</sup>CD8<sup>+</sup> double-positive T cell population, CD8 Naïve T cells (CD44<sup>+</sup>CD62<sup>-</sup>) were gated. The purity of CD8 Naïve T cells after sorting was as high as over 99.53%.
2. In vitro directional differentiation protocol for Tc cells: After the high-purity CD8 Naïve T cells after sorting were activated and cultured for 48 h with Mouse CD3/CD28 T Cell Activation Beads and IL-2 protein (20 U/mL), they can be further cultured for 96 h with active proteins IL-6 (50 ng/mL) and TGF-β (2 ng/mL) to be directionally differentiated into Tc1 and Tc17 cells respectively. Or they can be cultured for 96 h with active protein IL-4 (20 ng/mL) and mouse anti-IFN-γ (5 μg/mL) antibody to be directionally differentiated into Tc2 cells.

Fig. 37. Before and after the sorting of splenocytes from C57 mice, the purity of Naïve CD8 T cells (upper: before sorting; lower: after sorting) were detected using APC-CD3/Elab Fluor® Violet 450-CD8a/FITC-CD44/PE-CD62 Antibodies. Then, the cells were directionally differentiated using activation beads, cytokines and antibodies.



1. After high-purity CD8 Naïve T cells were sorted from the splenocytes of C57 mouse using a sorting kit, they were directionally differentiated into Tc1, Tc2, and Tc17 cells.
2. After the differentiated Tc cells were cultured with a stimulation blocker for 4 h for stimulation and blocking, detection was carried out. (The reagent used in this experiment was: Cell Stimulation and Protein Transport Inhibitor Kit (E-CK-A091)).
3. Phenotype of Tc1 cells: CD8<sup>+</sup>IFN-γ<sup>+</sup>; Phenotype of Tc2 cells: CD8<sup>+</sup>IL-4<sup>+</sup>; Phenotype of Tc17 cells: CD8<sup>+</sup>IL-17A<sup>+</sup>.

Fig. 38. Detection of the in vitro directional differentiation effect of mouse Naïve CD8 T cells.

Product Name	Cat. No.
APC Anti-Mouse CD3 Antibody[17A2]	E-AB-F1013E
Elab Fluor® Violet 450 Anti-Mouse CD8a Antibody[53-6.7]	E-AB-F1104Q
FITC Anti-Human/Mouse CD44 Antibody[IM7]	E-AB-F1100C
PE Anti-Mouse CD62L Antibody[MEL-14]	E-AB-F1011D
PE Anti-Mouse IFN-γ Antibody[XMG1.2]	E-AB-F1101D
APC Anti-Mouse IL-4 Antibody[11B11]	E-AB-F1204E
PE/Cyanine7 Anti-Mouse IL-17A Antibody[17F3]	E-AB-F1272H

## Regulatory T Cell Detection

Regulatory T cells (Tregs) are inhibitory cells that exert inhibitory functions on effector T cells (i.e., CD4<sup>+</sup> and CD8<sup>+</sup> cells) through a variety of different mechanisms. These mechanisms include the secretion of inhibitory cytokines such as TGF-β and IL-10, direct contact inhibition via the PD-1, CTLA-4, IDO, Tim-3, LAG-3 and ADO-PGE2 pathways, as well as the secretion of granzymes and other cytolytic molecules. The characteristic markers of regulatory T cells are listed in the following table:

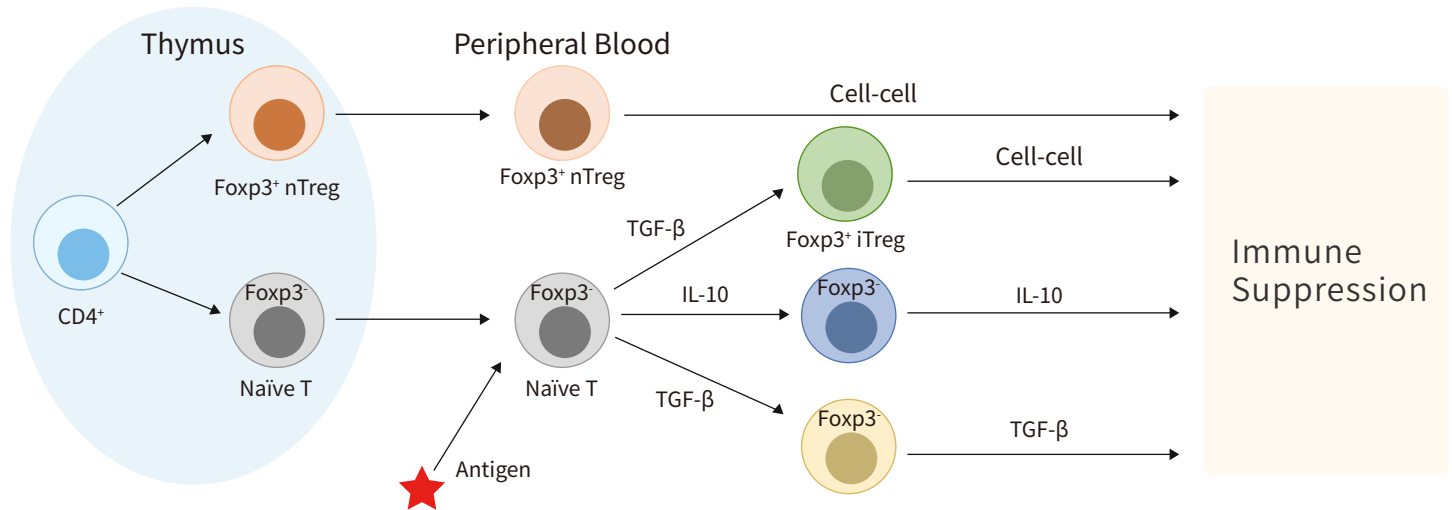


Fig. 39. Treg cell differentiation.

Cell Classification	Surface Marker	Secreted Cytokine
Naive Treg	CD4 <sup>+</sup> CD25 <sup>high</sup> CD127 <sup>-/low</sup> CD152 <sup>-</sup> Foxp3 <sup>low</sup> CD45RO <sup>-</sup>	TGF-β
Effector Treg	CD4 <sup>+</sup> CD25 <sup>high</sup> CD127 <sup>low</sup> CD152 <sup>+</sup> Foxp3 <sup>+</sup> CD45RO <sup>+</sup>	IL-10, TGFβ, IFN-γ, IL-17, CCL22, CXCL10
Terminal Effector Treg	CD4 <sup>+</sup> CD25 <sup>high</sup> CD127 <sup>-</sup> CD152 <sup>+</sup> Foxp3 <sup>+</sup> CD45RO <sup>+</sup>	IL-10, TGFβ

## Human Regulatory T Cell Detection

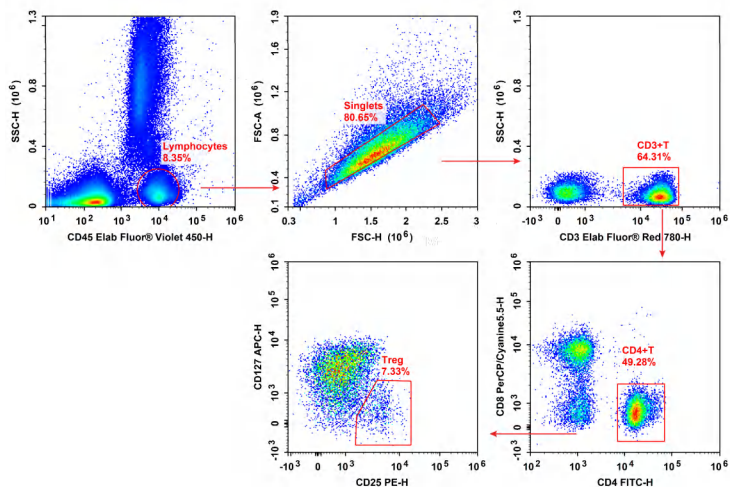


Fig. 40. Detection of Treg cells in human peripheral blood.

Product Name	Cat. No.
Elab Fluor® Red 780 Anti-Human CD3 Antibody[UCHT1]	E-AB-F1230S
FITC Anti-Human CD4 Antibody[SK3]	E-AB-F1352C
PerCP/Cyanine5.5 Anti-Human CD8a Antibody[OKT-8]	E-AB-F1110J
PE Anti-Human CD25 Antibody[BC96]	E-AB-F1194D
Elab Fluor® Violet 450 Anti-Human CD45 Antibody[HI30]	E-AB-F1137Q
APC Anti-Human CD127/IL-7RA Antibody[A019D5]	E-AB-F1152E

1. The traditional method of identifying Treg cells by detecting Foxp3 is relatively complicated to operate, and the steps of cell fixation and membrane permeabilization require a high level of proficiency from the experimenters. Currently, it is popular to detect human Treg cells using CD127 instead of Foxp3.
2. Use CD45 marker facilitates gating of lymphocytes. Lymphocyte gates can be directly defined by CD45 and SSC, and then the proportion of CD4<sup>+</sup>CD25<sup>+</sup>CD127<sup>-/low</sup> Treg cells can be analyzed. In the peripheral blood of normal individuals, Treg cells account for 3%-10% of lymphocytes.
3. It is recommended to set up single positive tubes to adjust compensation.

Mouse Regulatory T Cell Detection

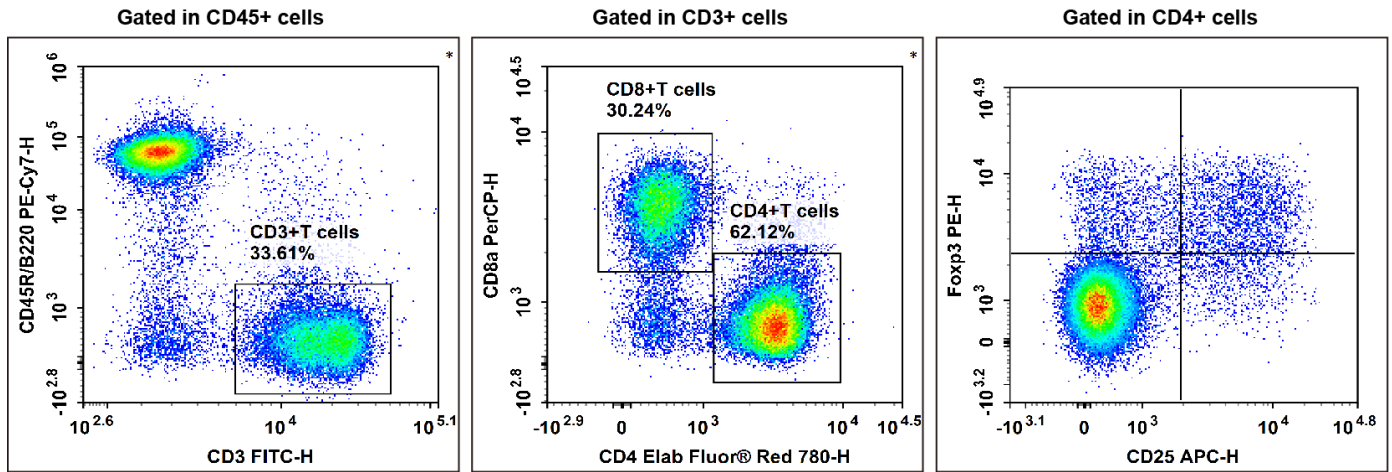


Fig. 41. Detection of Treg cells in mouse spleen.

Product Name	Cat. No.
FITC Anti-Mouse CD3 Antibody[17A2]	E-AB-F1013C
Elab Fluor® Red 780 Anti-Mouse CD4 Antibody[GK1.5]	E-AB-F1097S
PerCP Anti-Mouse CD8a Antibody[53-6.7]	E-AB-F1104F
APC Anti-Mouse CD25 Antibody[PC-61.5.3]	E-AB-F1102E
Elab Fluor® Violet 450 Anti-Mouse CD45 Antibody[30-F11]	E-AB-F1136Q
PE/Cyanine7 Anti-Mouse CD45R/B220 Antibody[RA3.3A 1/6.1]	E-AB-F1112H
PE Anti-Mouse/Rat Foxp3 Antibody[FJK-16s]	E-AB-F1351D

1. Phenotype of Mouse Treg cells: CD4<sup>+</sup>CD25<sup>+</sup>Foxp3<sup>+</sup>.
2. In this panel, it is recommended to set Isotype Control for CD25 and Foxp3, while other markers can be omitted due to obvious populations.
3. It is recommended to set up single positive tubes to adjust compensation.
4. Improper use of the membrane-permeabilizing agent can lead to high background and indistinct cell clustering.

■ Exhausted T Cell and Anergic T Cell Detection

**Exhausted T Cell:** T cell exhaustion is a state in which effector T cells lose their normal functions, typically caused by chronic infections and cancer. T cells, especially CD8<sup>+</sup> T cells, are chronically stimulated by pathogens causing chronic inflammation and tumor antigens. Gradually, they lose their original abilities to recognize antigens, activate and proliferate, and secrete interleukin-2 (IL-2). At the same time, they are inhibited by other regulatory T cells (Treg), ultimately resulting in the loss of their effector functions, the inability to differentiate into memory T cells (TM), and the failure to kill tumor cells or clear viruses. The most prominent features of exhausted T cells include the loss of their effector cytotoxic functions, changes in the expression of key transcription factors, up-regulation or co-expression of multiple inhibitory molecular receptors, and alterations in intracellular metabolism.

The main causes of T cell exhaustion are as follows: Defects in T cell function (increased expression of immune-allotypic receptors such as PD-1, CTLA4, and Tim-3), soluble immunosuppressive factors (such as an increase in IL-10 and IL-35), and interactions with other immune cells (such as the inhibitory activity of Treg cells), which together lead to T cell exhaustion.

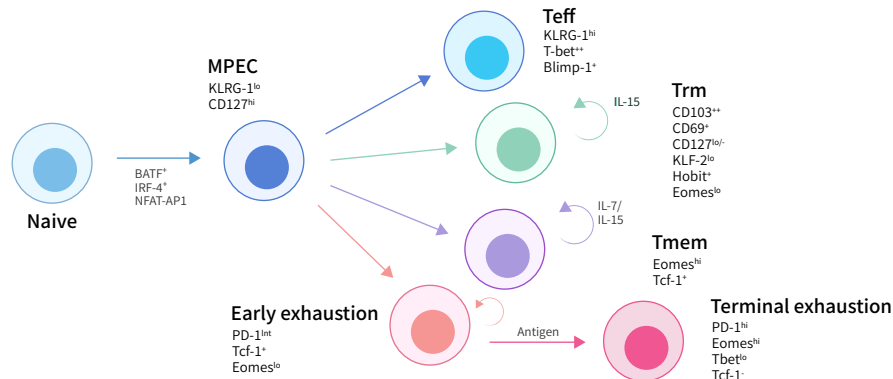
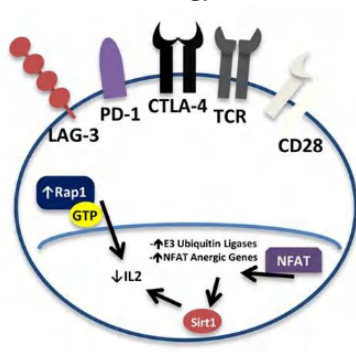


Fig. 42. T cell fates and phenotypes (DOI: 10.1146/annurev-immunol-041015-055318).

**Anergic T Cell:** The key role in anti-tumor immune responses is the T cell-mediated specific anti-tumor immune response. T cell activation is stimulated by two signals. The first signal is generated when the T cell receptor (TCR) recognizes the antigen peptide-major histocompatibility complex (MHC) molecule complex. The second signal is the co-stimulatory signal (CD28) binding to its corresponding receptor, which leads to T cell activation. If the TCR, as the first signal for T cells, lacks an appropriate co-stimulatory signal, T cell activation is inhibited, resulting in T cell apoptosis and T cell unresponsiveness upon re-stimulation by the antigen, which is also known as T cell anergy.

### Anergic T Cells

- Induced non-responsive state as part of peripheral tolerance.
- Low IL-2 production.
- Long-lived cells (immunosuppressive role?).



### Exhausted T Cells

- Unresponsive state-loss of effector functions.
- Long-lived and cell cycle arrested.
- Accumulate due to chronic infection or disease.
- Stable expression of inhibitory receptors.
- Layered co-inhibition (in function of repeated-activation).

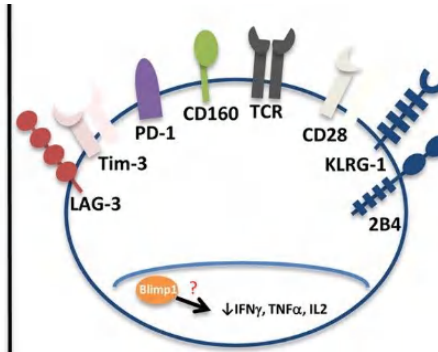


Fig. 43. General characteristic for anergic and exhausted T cells.

### Classical Panel

Application	Marker	Clone No.	Fluorochrome	Cat. No.
Detection of Inhibitory Receptor Expression on T Cells	CD3	UCHT1	FITC	E-AB-F1230C
	CD279/PD-1	EH12.2H7	PE	E-AB-F1229D
Detection of Activating Receptor Expression on T Cells	CD3	UCHT1	FITC	E-AB-F1230C
	CD16/56	3G8	PE	E-AB-F1236D
	CD56	5.1H11	PE	E-AB-F1239D

Cell Classification	Surface Marker	Transcription Factor
Exhausted T	CD3, CD8, CD279/PD-1, CD274/PD-L1, CD366/Tim-3, 1B11, LAG3, CTLA4	BLIMP1
Anergic T	αβTCR, CD3, BTLA	GRAIL, CBL-B, ITCH, NEDD4

### Human Exhausted T Cell Detection

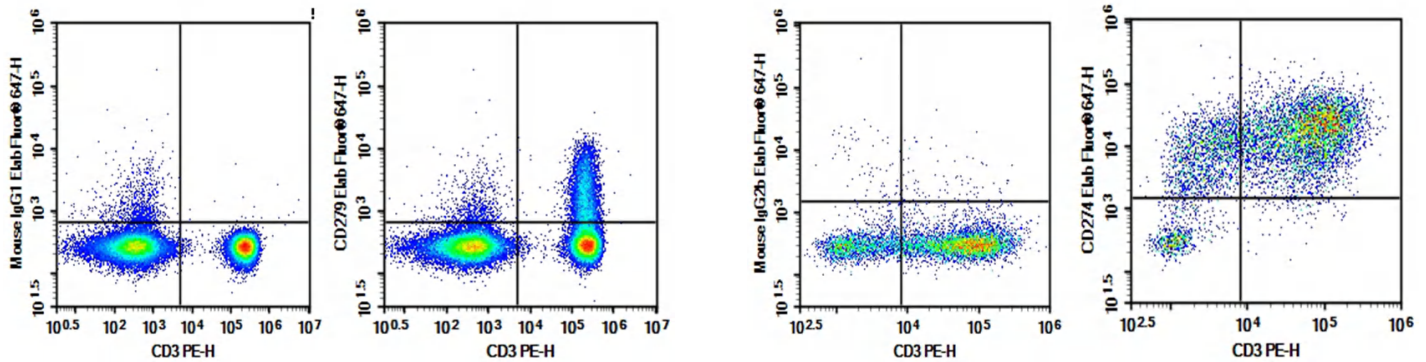


Fig. 44. Detection of human exhausted T cells.

Product Name	Cat. No.
PE Anti-Human CD3 Antibody[UCHT1]	E-AB-F1230D
AF/LE Purified Anti-Human CD274/PD-L1 Antibody[29E.2A3]	E-AB-F11330
AF/LE Purified Anti-Human CD279 Antibody[EH12.2H7]	E-AB-F12290

1. For human peripheral blood (PBMC), detect the expression of PD-1/CD279 in CD3<sup>+</sup> T cells to analyze the activation and exhaustion status of T cells. After T cell activation, the expression of PD-1 was upregulated. Long-term antigen stimulation (such as chronic viral infection or tumor) will lead to T cell "exhaustion". At this time, PD-1 is continuously highly expressed (it can exceed 30%) and may be accompanied by functional defects (such as decreased cytotoxicity and reduced cytokine secretion).
2. Human peripheral blood (PBMC), stimulate and culture them with 10 μg/mL PHA for 3 days. Then, detect and analyze the expression of PD-L1/CD274 in CD3<sup>+</sup> T cells to analyze the immunosuppressive state of activated T cells. T cells autonomously regulate their own activity through the expression of PD-L1 to avoid over-activation or functional exhaustion.

Mouse Exhausted T Cell Detection

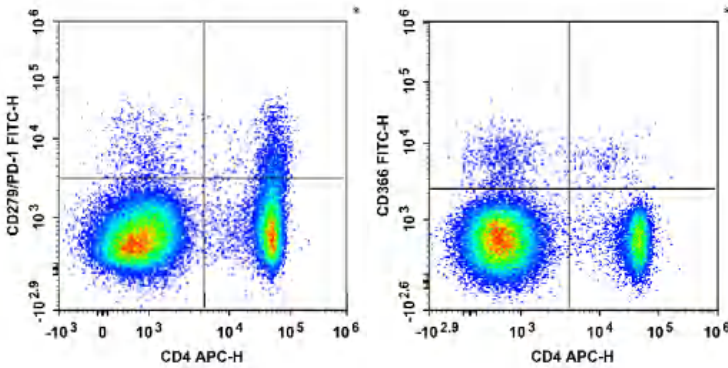


Fig. 45. Detection of mouse exhausted T cells.

Product Name	Cat. No.
APC Anti-Mouse CD4 Antibody[GK1.5]	E-AB-F1097E
FITC Anti-Mouse CD279/PD-1 Antibody[29F.1A12]	E-AB-F1131C
FITC Anti-Mouse CD366/Tim-3 Antibody[RMT3-23]	E-AB-F1192C

1. After lysing red blood cells from the spleen cells of C57 mouse using ACK Lysis Buffer, detect and analyze the exhaustion status of CD4<sup>+</sup> T cells using CD4/CD366 (Tim-3)/PD-1 (CD279) Antibodies.
2. Detecting the proportions of the CD4<sup>+</sup>PD-1<sup>+</sup> T cell population and the CD4<sup>+</sup>CD366 (Tim-3)<sup>+</sup> T cell population can help analyze the dynamic differentiation pathway and the mechanism of functional decline of CD4<sup>+</sup> T cells under chronic antigen stimulation.

■ Literature Citations for T Cell Research Products (partial)

Elabscience® products have been cited in over **28,500** research literature, with a combined impact factor exceeding **150,000**. The journals where the citations were published include *Cell Metabolism*, *Cell Reports Medicine*, *Nature Cancer*, *Nature Communications*, *Nature Immunology*, *Nature Materials*, *Nature Nanotechnology*, *Science Advances*, *Science Translational Medicine*, etc. The institutions where the research was conducted and published include Johns Hopkins University, the University of Michigan in the United States, and the Max Planck Institute for Biology in Tübingen, Germany as well as Tsinghua University and Peking University. On the journey of T cell exploration, Elabscience® has always been your reliable partner. We are committed to providing solid data support for your research breakthroughs with our high-quality products. The following literature showcases the applications of our products in T cell research:

1. Si X, Shao M, Teng X, et al. Mitochondrial isocitrate dehydrogenase impedes CAR T cell function by restraining antioxidant metabolism and histone acetylation. *Cell Metabolism*. 2024;36(1):176-192.e10.
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## 07 More Recommendations for T Cell Detection

### ■ Recommended Products for Human T Cell Detection Panel

Application	Marker	Clone No.	Fluorochrome	Cat. No.
Human T Cells Panel (4-color)	CD45	HI30	PerCP/Cyanine5.5	E-AB-F1137J
	CD3	OKT-3	APC	E-AB-F1001E
	CD4	SK3	FITC	E-AB-F1352C
	CD8	OKT-8	PE	E-AB-F1110D
Human T Cell Panel in Peripheral Blood (8-color)	CD45	HI30	APC/Cyanine7	E-AB-F1137N
	CD3	UCHT1	Elab Bright™ Violet 510	E-AB-F1230R1
	CD4	SK3	Elab Fluor® Violet 450	E-AB-F1352Q
	CD8	SK-1	PerCP/Cyanine5.5	E-AB-F1110J
	CD45RA	HI100	PE/Cyanine7	E-AB-F1052H
	CD45RO	UCHL1	APC	E-AB-F1139E
	CD127	A019D5	FITC	E-AB-F1152C
	CD25	CHI621	PE	AN00360D
Human Naïve/Memory T Cell Panel (4-color)	CD3	OKT-3	Elab Fluor® 700	E-AB-F1001M1
	CD4	SK3	PerCP/Cyanine5.5	E-AB-F1109J
	CD45RA	HI100	FITC	E-AB-F1052C
	CD197	G043H7	APC	E-AB-F1159D
Human Naïve/Memory T Cell Panel (4-color)	CD3	OKT-3	APC	E-AB-F1001E
	CD4	SK3	PE	E-AB-F1352D
	CD45RA	HI100	PerCP	E-AB-F1052F
	CD45RO	UCHL1	FITC	E-AB-F1139C
Human T/NK Cell Panel (5-color)	CD45	HI30	PE/Cyanine7	E-AB-F1137H
	CD3	UCHT1	FITC	E-AB-F1230C
	CD4	SK3	PerCP	E-AB-F1352F
	CD8	OKT-8	APC	E-AB-F1110E
	CD56	5.1H11	Elab Fluor® Red 780	E-AB-F1239S
Human T/B/NK Cell Panel (4-color)	CD45	HI30	PerCP	E-AB-F1137F
	CD3	OKT-3	FITC	E-AB-F1001C
	CD19	CB19	APC	E-AB-F1004E
	CD16	3G8	PE	E-AB-F1236D
	CD56	5.1H11	PE	E-AB-F1239D
Human TN/TEM/TN/TSEM/TCM Cell Panel (6-color)	CD3	OKT-3	FITC	E-AB-F1001C
	CD4	SK3	PerCP	E-AB-F1352F
	CD8	OKT-8	APC	E-AB-F1110E
	CD45RO	UCHL1	PE	E-AB-F1052D
	CD197/CCR7	G043H7	APC	E-AB-F1159E
	CD62L	DX2	PE/Cyanine7	E-AB-F1168H

Application	Marker	Clone No.	Fluorochrome	Cat. No.
Human T Cell Inhibitory Receptor Expression (2-color)	CD3	OKT-3	FITC	E-AB-F1001C
	PD-1	EH12.2H7	PE	E-AB-F1229D
Human T Cell Activation Status Detection (4-color)	CD3	UCHT1	PerCP/Cyanine5.5	E-AB-F1230J
	CD69	FN50	PE	E-AB-F1138D
	CD25	BC96	FITC	E-AB-F1194C
	HLA-DR	L243	APC	E-AB-F1111E
Human Th Cell Panel (7-color)	CD45	HI30	Elab Fluor® Violet 540	E-AB-F1137T3
	CD4	RPA-T4	Elab Fluor® Violet 450	E-AB-F1109Q
	CD3	OKT-3	PerCP/Cyanine5.5	E-AB-F1001J
	CXCR3	G025H7	APC	E-AB-F1156E
	CCR4	L291H4	Elab Fluor® 700	E-AB-F1366M1
	CCR6	G034E3	PE	E-AB-F1158D
	CXCR5	J252D4	FITC	E-AB-F1287C
Human Th1/Th2 Cell Panel (4-color)	CD3	OKT-3	PerCP/Cyanine5.5	E-AB-F1001J
	CD4	SK3	Elab Fluor® 488	E-AB-F1352L
	IFN-γ	B27	APC	E-AB-F1196E
	IL-4	MP4-25D2	PE	E-AB-F1203D
Human Th17 Cell Panel (3-color)	CD3	OKT-3	PerCP/Cyanine5.5	E-AB-F1001J
	CD4	SK3	Elab Fluor® 488	E-AB-F1352L
	IL-17A	BL168	PE	E-AB-F1173D
Human Treg Cell Panel (3-color)	CD4	SK3	FITC	E-AB-F1352C
	CD25	BC96	PE	E-AB-F1194D
	CD127	A019D5	APC	E-AB-F1152E
Human Treg Cell Panel (6-color)	CD45	HI30	Elab Fluor® Violet 450	E-AB-F1137Q
	CD3	UCHT1	Elab Fluor® Red 780	E-AB-F1230S
	CD4	SK3	FITC	E-AB-F1352C
	CD8	OKT-8	PerCP/Cyanine5.5	E-AB-F1110J
	CD25	BC96	PE	E-AB-F1194D
	CD127	A019D5	APC	E-AB-F1152E
Human CAR-T Immune Function TN/TEM/TCM Cell Panel (8-color)	CD8	OKT-8	Elab Fluor® Violet 610	E-AB-F1110T
	CCR7/CD197	G043H7	Elab Fluor® Violet 450	E-AB-F1159Q
	CD3	UCHT1	FITC	E-AB-F1230C
	CD4	SK3	PerCP	E-AB-F1352F
	CD45RA	HI100	PE/Cyanine7	E-AB-F1052H
	CD45	HI30	APC/Cyanine7	E-AB-F1137N
	CD28	CD28.2	APC	E-AB-F1195E
	CD69	FN50	PE	E-AB-F1138D
Human Allergy Detection (3-color)	CD3	OKT-3	FITC	E-AB-F1001C
	CD19	CB19	PE	E-AB-F1004D
	CD23	EBVCS2	APC	E-AB-F1382E
Human Tumor Metastasis Related Detection	CD4	RPA-T4	FITC	E-AB-F1109C
	CXCR4/CD184	12G5	PE	E-AB-F1157D

Application	Marker	Clone No.	Fluorochrome	Cat. No.
Human NK Cell Panel (4-color)	CD16	3G8	FITC	E-AB-F1236C
	HLA-DR	L243	PE	E-AB-F1111D
	CD3	OKT-3	PerCP/Cyanine5.5	E-AB-F1001J
	CD56	B-A19	APC	E-AB-F1305E

## ■ Recommended Products for Mouse T Cell Detection Panel

Application	Marker	Clone No.	Fluorochrome	Cat. No.
Mouse T Cells Panel (4-color)	CD45	30-F11	PerCP/Cyanine5.5	E-AB-F1136J
	CD3	17A2	APC	E-AB-F1013E
	CD4	GK1.5	FITC	E-AB-F1097C
	CD8	53-6.7	PE	E-AB-F1104D
Mouse Naïve/Memory T Cell Panel (4-color)	CD3	17A2	APC	E-AB-F1013E
	CD4	GK1.5	Elab Fluor® Violet 450	E-AB-F1097Q
	CD44	IM7	FITC	E-AB-F1100C
	CD62L	MEL-14	PE	E-AB-F1011D
Mouse Naïve/Memory T Cell Panel (5-color)	CD3	17A2	PerCP/Cyanine5.5	E-AB-F1013J
	CD4	GK1.5	Elab Fluor® Violet 450	E-AB-F1097Q
	CD8	53-6.7	APC	E-AB-F1104E
	CD44	IM7	FITC	E-AB-F1100C
	CD62L	MEL-14	PE	E-AB-F1011D
Mouse T/B/NK Cell Panel (6-color)	CD45R/B220	RA3.3A 1/6.1	FITC	E-AB-F1112C
	CD45	30-F11	PerCP/Cyanine5.5	E-AB-F1136J
	CD3	17A2	Elab Fluor® Violet 450	E-AB-F1013Q
	CD4	GK1.5	PE/Cyanine7	E-AB-F1097H
	CD8	53-6.7	APC	E-AB-F1104E
	NK1.1	PK136	PE	E-AB-F0987D
Mouse TN/TEM/TN/TSEM/TCM Cell Panel (6-color)	CD3	17A2	FITC	E-AB-F1013C
	CD4	GK1.5	PerCP/Cyanine5.5	E-AB-F1097J
	CD8	53-6.7	PE	E-AB-F1104D
	CD44	IM7	PE/Cyanine7	E-AB-F1100H
	CD45	30-F11	Elab Fluor® Violet 450	E-AB-F1136Q
	CD62L	MEL-14	APC	E-AB-F1011E
Mouse T Cell Activation State Cell Panel (4-color)	CD3	17A2	PerCP/Cyanine5.5	E-AB-F1013J
	CD69	H1.2F3	PE	E-AB-F1187D
	CD25	PC-61.5.3	FITC	E-AB-F1102C
	CD38	NIMR5	APC	E-AB-F1193E
Mouse Th1/Th2 Cell Panel (4-color)	CD3	17A2	PerCP/Cyanine5.5	E-AB-F1013J
	CD4	RM4-5	FITC	E-AB-F1353C
	IFN- $\gamma$	XMG1.2	APC	E-AB-F1101E
	IL-4	11B11	PE	E-AB-F1204D
Mouse Th17 Cell Panel (3-color)	CD3	17A2	PerCP/Cyanine5.5	E-AB-F1013J
	CD4	RM4-5	FITC	E-AB-F1353C
	IL-17A	TC11-18H10.1	PE	E-AB-F1199D

Application	Marker	Clone No.	Fluorochrome	Cat. No.
Mouse Treg Cell Panel (7-color)	Foxp3	FJK-16s	PE	E-AB-F1351D
	CD45R/B220	RA3.3A 1/6.1	PE/Cyanine7	E-AB-F1112H
	CD25	PC-61.5.3	APC	E-AB-F1102E
	CD4	GK1.5	Elab Fluor® Red 780	E-AB-F1097S
	CD45	30-F11	Elab Fluor® Violet 450	E-AB-F1136Q
	CD3	17A2	FITC	E-AB-F1013C
	CD8a	53-6.7	PerCP	E-AB-F1104F

## ■ Recommended Products for T Cell Metabolism and Health Detection

Application	Product Name	Cat. No.
T Cell Metabolic Function Detection	ATP/ADP Ratio Chemiluminescence Assay kit	E-BC-F004
	Enhanced ATP Chemiluminescence Assay Kit	E-BC-F201
	Enhanced Oxygen Consumption Rate (OCR) Fluorometric Assay Kit	E-BC-F070
	Extracellular Acidification Rate (ECAR) Fluorometric Assay Kit	E-BC-F069
	Fatty Acid Oxidation (FAO) Colorimetric Assay Kit	E-BC-K784-M
	Free Fatty Acids (NEFA/FFA) Fluorometric Assay Kit	E-BC-F039
	Glutamine (Gln) Colorimetric Assay Kit	E-BC-K853-M
	Glucose (GLU) Fluorometric Assay Kit	E-BC-F037
	Glycolysis Stress Fluorometric Assay Kit	E-BC-F084
	Glucose Uptake Fluorometric Assay Kit	E-BC-F041
	Mitochondrial Stress Fluorometric Assay Kit	E-BC-F078
	Pyruvate Fluorometric Assay Kit	E-BC-F058
	2-NBDG Glucose Uptake Cell-Based Kit	E-CK-A441
T Cell Lactate Metabolism Detection	Human D-LDH (D-Lactate Dehydrogenase) ELISA Kit	E-EL-H0866
	Human LDHA (Lactate Dehydrogenase A) ELISA Kit	E-EL-H0556
	Lactate Dehydrogenase (LDH) Activity Assay Kit (WST-8 method)	E-BC-K766-M
T Cell Immunosuppression Detection	Human CTLA4(Cytotoxic T-Lymphocyte Associated Antigen 4) ELISA Kit	E-EL-H2069
	Mouse CTLA4(Cytotoxic T-Lymphocyte Associated Antigen 4) ELISA Kit	E-EL-M0398
Cell Apoptosis Detection	Annexin V-FITC/PI Apoptosis Kit	E-CK-A211
	Annexin V-APC/7-AAD Apoptosis Kit	E-CK-A218
	Caspase 1 Activity Detection Substrate for Flow Cytometry	E-CK-A481
	Caspase 3/7 Activity Detection Substrate for Flow Cytometry	E-CK-A483
	Caspase 4 Activity Detection Substrate for Flow Cytometry	E-CK-A484
	Caspase 6 Activity Detection Substrate for Flow Cytometry	E-CK-A486
	Caspase 8 Activity Detection Substrate for Flow Cytometry	E-CK-A488
	Caspase 9 Activity Detection Substrate for Flow Cytometry	E-CK-A489
	One-step TUNEL In Situ Apoptosis Kit (Green, FITC)	E-CK-A320
One-step TUNEL In Situ Apoptosis Kit (Red, Elab Fluor® 594)	E-CK-A322	

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