# Human Th17 Flow Cytometry Staining Kit

**Cat. No: XJH002** **Size: 20 Assays/100 Assays**

## Kit Components

| Cat. | Products | 20 Assays | 100 Assays | Storage |
|---|---|---|---|---|
| XJH002A | Human Th17 Cytokine Detection Antibody Cocktail | 200 μL | 1 mL | 2~8 ℃, shading light |
| XJH002B | Human Th17 Cytokine Detection Antibody Isotype Cocktail | 200 μL | 1 mL | 2~8 ℃, shading light |
| E-CK-A011 | Cell Stimulation MIX Powder (50 μg) | 50 μg | 50 μg×3 | -20 ℃, shading light |
| E-CK-A012 | Cell Stimulation MIX Solvent | 120 μL | 360 μL | -20 ℃, shading light |
| E-CK-A013 | Protein Transport Inhibitor MIX Powder (200 μg) | 200 μg | 200 μg×3 | -20 ℃, shading light |
| E-CK-A109A | Fixation Buffer | 10 mL | 10 mL | 2~8 ℃ |
| E-CK-A109B | Permeabilization Buffer (5×) | 15 mL | 50 mL | 2~8 ℃ |
| | Manual | | One Copy | |

## Composition of Components

| Products | Component |
|---|---|
| Human Th17 Cytokine Detection Antibody Cocktail | PerCP/Cyanine5.5 Anti-Human CD3 Antibody[OKT3] |
| | Elab Fluor® 488 Anti-Human CD4 Antibody[SK3] |
| | PE Anti-Human IL-17A Antibody[BL168] |
| Human Th17 Cytokine Detection Antibody Isotype Cocktail | PerCP/Cyanine5.5 Anti-Human CD3 Antibody[OKT3] |
| | Elab Fluor® 488 Anti-Human CD4 Antibody[SK3] |
| | PE Mouse IgG1,κ Isotype Control[MOPC-21] |

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

## Storage

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

## Introduction

The Elabscience® Human Th17 Flow Cytometry Staining Kit can be used to detect the proportion of Th17 cells among T lymphocytes (or $CD4^+$ helper T cells) in human anticoagulants.. Th cells, also known as helper T cells (helper T cells), have a very weakly ability to differentiate from Th0 to Th1, Th2, and Th17 cells in the resting state (unstimulated state, e.g. normal physiological state in human). There are only a very small number of Th1,


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Th2 and Th17 cells in the peripheral blood, when the Th cells are stimulated by external factors (e.g, stimuli, pathogens, etc.), the Th0 will differentiate into Th1, Th2 or Th17, and the specific tendency of the differentiation will depend on the type of cytokine in the microenvironment. The proportion of Th1, Th2 or Th17 cells in the activated T lymphocytes can be known by detecting the expression of IFN-γ, IL-4 or IL-17A in the sample. This product provides a mixture of three-color fluorescently labeled antibodies that specifically bind to human CD3, CD4, IL-17A (for detection of Th17), allowing for the detection of Th17 by multicolor panel.

Activated cells are stimulated to secrete cytokines by Cell Stimulation MIX in the kit, and the induced secretion of cytokines is further blocked intracellularly by the Protein Transport Inhibitor MIX. The activated T cells were fixed by Fixation Buffer and then permeabilized with Permeabilization Buffer to fully permeabilize the cell membrane, and the permeabilization could be synchronized with the binding of cell surface/intracellular antigens and antibodies, and finally the intracellular secretion of cytokines from the activated T cells was detected by flow cytometry. The surface markers of the Th cells are $CD3^+CD4^+$, and the correct gate was selected for $CD4^+$ T cells, and the proportion of Th17 cells in the activated T lymphocytes was further analyzed by detecting the fluorescence signal on IL-17A antibody.

## Materials Not Supplied

- **Reagents**

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

- **Instruments**

Flow cytometer, $CO_2$ incubator, centrifuge.

## Reagent Preparation

**1) 500× Cell Stimulation MIX**

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

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

**2) 1000× Protein Transport Inhibitor MIX**

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

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

**3) 1× Permeabilization Buffer**

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

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

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

**4) RPMI-1640 complete medium**


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

## Experimental Protocol

➢ **For human peripheral blood mononuclear cell (PBMC) sample**

**Note: Sterile technique is required for the step 1-6.**

1. Collect 10 mL of fresh human blood anticoagulated with heparin sodium and proceed to the cell room for operations. The entire isolation process must be conducted under sterile conditions.
   Note: The PBMC separation operation should be carried out within 1 hour after collecting fresh human blood. Approximately $1\times10^7$ PBMCs can be obtained from 10 mL of human blood. The Ficoll separation and centrifugation process should be carried out at 20±2 ℃. Higher temperatures may reduce cell yield, while lower temperatures may result in incomplete separation and reduced purity.
2. Add 5 mL of ficoll lymphocyte separation solution (self-prepared) to two 15mL centrifuge tubes, respectively. Then slowly add 5 mL of fresh human blood along the tube wall (mix the blood thoroughly before adding). Do not mix or shake the centrifuge tubes after adding the blood. Centrifuge at 350×g for 20 min.
3. Discard the top layer of supernatant (approximately 1~2 mL). Aspirate the second layer of supernatant and a small amount of the third layer of separation solution, and transfer it to a 50 mL centrifuge tube.
4. Add 20 mL of RPMI-1640 basal medium (without serum) and mix fully. Centrifuge at 250×g for 5 min, then discard the supernatant.
   Note: The cell pellet is generally grayish-white. If it appears red, it indicates the presence of residual erythrocyte. You can either use it directly or add an appropriate amount of red blood cell lysis buffer to lyse the red blood cells and obtain the target cells.
5. Resuspend the cells in an appropriate amount of RPMI-1640 complete medium and count the cells. Adjust the cell density to $1\times10^6$ cells/mL with the complete medium and seeded.
6. Refer to the table below to add Cell Stimulation MIX and Protein Transport Inhibitor MIX. Incubate in a 37 ℃, 5% $CO_2$ incubator for 5 hours, mix every 1~2 hours.

| Group | Experimental Content | Addition of Antibodies |
|---|---|---|
| Negative group | Untreated | XJH002A |
| Test group | Add 2 μL of Cell Stimulation MIX (500×) and 1 μL of Protein Transport Inhibitor MIX (1000×) to each 1 mL of cell suspension | |
| Isotype group | | XJH002B |

**In the subsequent steps, sterile technique is not necessary.**

7. Collect the cells by centrifuging at 300×g for 5 min. Discard the supernatant and resuspend the cells in 1 mL of Cell Staining Buffer or 1× PBS. Centrifuge again at 300×g for 5 min and discard the supernatant.
8. Each $1\times10^6$ cells were resuspended with 180 μL of Cell Staining Buffer or 1× PBS, then add 60 μL of Fixation Buffer and mix thoroughly. Fix the cells overnight at 4 ℃ or at room temperature for 1 hour.
9. After fixation, the cells will settle at the bottom of the EP tube. Discard the supernatant and add 1~2 mL of 1× Permeabilization Buffer. Mix fully and centrifuge at 500×g for 5 min, and discard the supernatant.
10. Resuspend the cells in 100 μL of 1× Permeabilization Buffer. Add 10 μL of the antibody to each group according to the table above. Incubate at room temperature with shading light for 60 min, mix every 15~20 min.

11. After incubation, add 1~2 mL of Cell Staining Buffer or 1× PBS. Mix fully and centrifuge at 500×g for 5 min, and discard the supernatant.
12. Resuspend the cells in 100~200 µL of Cell Staining Buffer or 1× PBS and detect the cytokine expression with flow cytometry.

| Detection indicators | Fluorophore | Ex (nm) | Em (nm) |
|---|---|---|---|
| CD3 | PerCP/Cyanine5.5 | 440, 480, 675 | 675 |
| CD4 | Elab Fluor® 488 | 495 | 520 |
| IL-17A | PE | 495, 565 | 575 |

➢ **For human whole blood sample**

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

1. Collect fresh human blood anticoagulated with heparin sodium, Add 250 µL of anticoagulated blood and 250 µL of RPMI-1640 basal medium (without serum) to each group and mix fully.
2. Refer to the table below to add Cell Stimulation MIX and Protein Transport Inhibitor MIX according to the table below. Incubate at 37 ℃ in a 5% CO2 incubator for 5 hours, mix every 1-2 hours.

| Group | Experimental Content | Addition of Antibodies |
|---|---|---|
| Negative group | Untreated | XJH002A |
| Test group | Add 1 µL Cell Stimulation MIX (500×) and 0.5 µL Protein Transport Inhibitor MIX (1000×) | |
| Isotype group | | XJH002B |

**In the subsequent steps, sterile technique is not necessary.**

3. Transfer the cells to 2 mL EP tubes. Add 1 mL of Cell Staining Buffer or 1× PBS and mix fully. Centrifuge at 300×g for 5 min and discard the supernatant.
4. Resuspend the cells in 100 µL of Cell Staining Buffer or 1× PBS. Then add 2 mL of 1× ACK Lysis Buffer (E-CK-A105) and lyse on ice for 2-3 min. Centrifuge at 300×g for 5 min.
   Note: Lysis is complete when the solution turns from cloudy to clear. Centrifuge promptly to avoid over-lysis and potential cell damage.
5. Resuspend the cells in 1 mL of Cell Staining Buffer or 1× PBS. Centrifuge at 300×g for 5 minutes and discard the supernatant.
6. Resuspend the cells in 180 µL of Cell Staining Buffer or 1× PBS. Add 60 µL of Fixation Buffer and mix thoroughly. Fix the cells overnight at 4 ℃ or at room temperature for 1 hour.
7. After fixation, the cells will settle at the bottom of the EP tube. Discard the supernatant. Add 1~2 mL of 1× Permeabilization Buffer, mix fully, and centrifuge at 500×g for 5 min. Discard the supernatant.
8. Resuspend the cells in 100 µL of 1× Permeabilization Buffer. Add 10 µL of the antibody to each group according to the table above. Incubate at room temperature with shading light for 60 min, mix every 15~20 min.
9. After incubation, add 1 mL of Cell Staining Buffer or 1× PBS. Mix fully and centrifuge at 500×g for 5 min. Discard the supernatant.

10. Resuspend the cells in 100~200 μL of Cell Staining Buffer or 1× PBS and detect the cytokine expression with flow cytometry.

| Detection indicators | Fluorophore | Ex (nm) | Em (nm) |
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| CD3 | PerCP/Cyanine5.5 | 440, 480, 675 | 675 |
| CD4 | Elab Fluor® 488 | 495 | 520 |
| IL-17A | PE | 495, 565 | 575 |

## Cautions

1. This product is for research use only.
2. Permeabilization Buffer (5×) may cause precipitation, which is normal and does not affect the effectiveness.
3. When collecting fresh human whole blood samples, it is recommended to use heparin sodium as an anticoagulant. EDTA or similar calcium-chelating anticoagulants may affect cytokine secretion.
4. For optimal results, separate PBMCs from fresh human blood within 1 hour of collection. The Ficoll gradient centrifugation should be performed at 20±2℃. Higher temperatures may reduce cell yield, while lower temperatures may lead to incomplete separation and reduced purity.
5. During the PBMC separation process, the blood should be thoroughly mixed before be added to ficoll lymphocyte separation solution. When adding the blood, it is important to avoid disturbing the layered liquid surface to ensure a good interface is formed, otherwise it may affect the separation results.
6. High acceleration and deceleration speeds during centrifugation can cause cell loss. It is recommended to set the acceleration to no more than 3 and the deceleration to no more than 2 (i.e., Acc≦3, Dec≦2).
7. Fluorescent substances are prone to quenching. Protect them from light during handling and storage.
8. For your safety and health, please wear laboratory overalls and disposable gloves for operation.

## Common Problems and Solutions

| Common Problem | Possible Cause | Suggestion |
|---|---|---|
| Contamination of lymphocyte layer with red blood cells | Experimental temperature were too cold. | Allow samples and reagents to reach 20±2 ℃ before starting the experiment. |
| | Centrifugal speed was too low. | Increase the centrifugation speed appropriately. |
| | Centrifugation time was too short. | Increase the centrifugation time appropriately. |
| Low lymphocyte viability and yield | Excessive temperature | Allow samples and reagents to reach 20±2 ℃ before starting the experiment. |
| | The storage time of blood was too long. | Use fresh blood for separation to ensure lymphocyte viability. |
| Contamination with granulocytes, etc. | Centrifuge was not balanced during centrifugation. | The centrifuge should be leveled before use. |
| | Sudden stop of the centrifuge causing mixing of lymphocyte layer and lower layers. | Set the centrifuge to decelerate slowly (Dec≦2) to allow gradual stopping. |
| No cytokine expression | Excessive cell density. | Adjust cell density to $1\sim2\times10^6$ cells/mL. |


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| | | |
|---|---|---|
| detected | Interference from red blood cells. | Use human peripheral blood lymphocyte separation medium correctly or use a red blood cell lysis method to eliminate interference. |
| | Reagents expired. | Store reagents properly and use them within expiration date. |
| | Poor cell fixation/permeabilization. | Control fixation/permeabilization time according to the instructions. |
| | Insufficient induction time. | Conduct a preliminary experiment by setting gradient induction time to explore the optimal reaction time. |
| Excessive intracellular cytokine expression | Poor cell condition, high number of dead cells. | Ensure cells are in good condition prior to induction and exclude dead cells. |
| | Non-specific antibody binding. | Increase antibody blocking steps to reduce non-specific staining. |
| Weak fluorescence signal in flow cytometry | Insufficient amount of antibody used. | Add the antibody according to the instructions. |
| | Insufficient antibody staining time. | Extend antibody incubation time according to the instructions or as needed. |
| | Too many cells. | Reduce cell density. |
| | Very low target protein expression level. | Enrich target cells before induction and detection. |

## Typical results

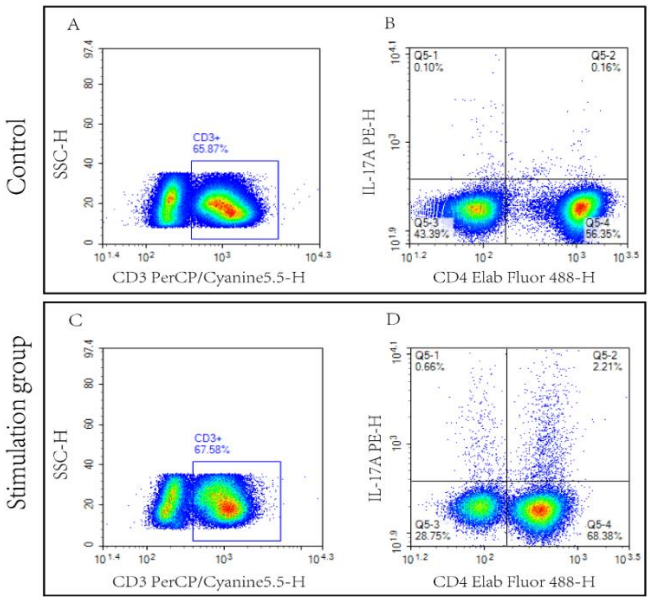


**Figure 1**: Flow cytometric analysis of Human Th17 Flow Cytometry Staining Kit. Normal PBMC cells (Control) and PBMC cells stimulated with Cell Stimulation MIX(Stimulation group). $CD3^+$ $CD4^+$ $IL\text{-}17A^+$cells (Q5-2) were gated to analyze Th17.


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