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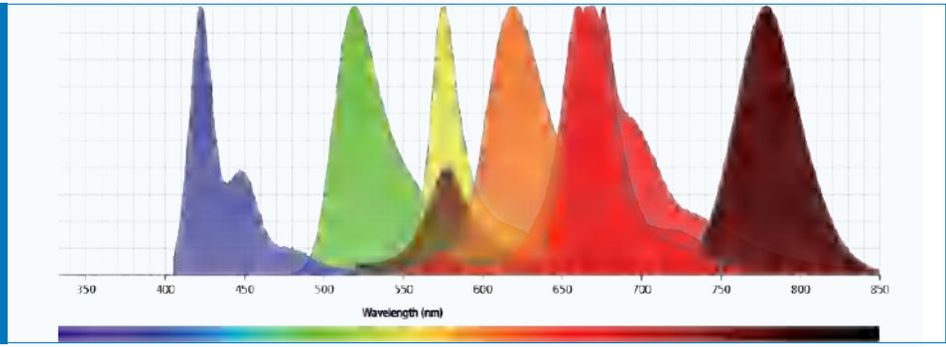
Sample Preparation Guide for Flow Cytometry Experiment



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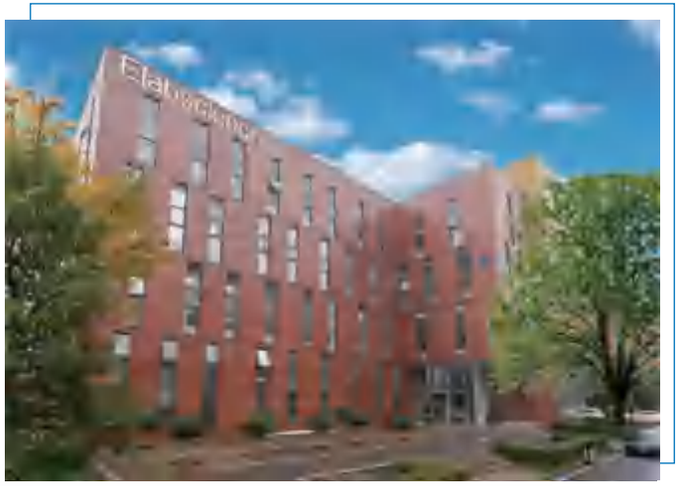
ABOUT US



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

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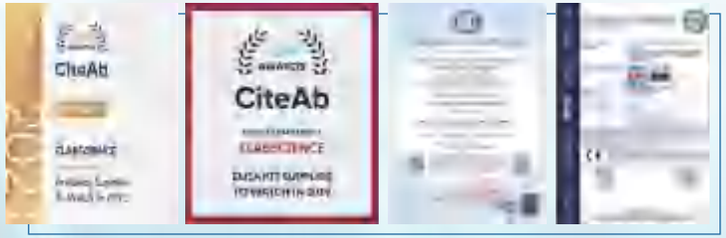

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Sample Preparasion Method and Precaustions for Flow Cytometry Experiment

- Flow cytometry is a fascinating experiment, which is high-speed, objective, stable and statistically significant. It can handle complex samples and obtain a variety of parameters at the same time, quickly determine the biological properties of single cells in cell suspension, and can sort and collect specific cells. It is widely used in cell biology, immunology, communication, basic medicine and other fields.
- The analysis and detection of flow cytometry is based on a single cell. In order to obtain good flow experiment results, the sample must be prepared into a single cell suspension. The cell mass is easy to block the instrument, and too much cell debris will lead to unsatisfactory experimental results.
- The technical team of Elabsclence® has organized some common flow experiment sample preparation processes and precautions, and illustrated with cases to help customers better conduct flow experiments and obtain better experimental results.

Human Peripheral Blood PBMC Preparation Process and Precautions

The preparation process of human peripheral blood PBMC

01 Collect anticoagulated human peripheral blood, dilute it with 1640 medium or PBS at a ratio of 1:1, and mix upside down or by pipetting.

02 Add 3 mL of well-mixed Ficoll solution (1.077 density) to a 15 mL centrifuge tube, then add 2 mL of diluted blood along the tube wall. The obviously stratification of blood and Ficoll liquid represents successful preparation.

03 Transfer the sample to a centrifuge and centrifuge at 500 g for 25 min.

04 Take out the centrifuge tube, then discard the upper yellow liquid and suck up mononuclear cells in the middle white film layer.

05 Wash the obtained mononuclear cells with 10 mL of PBS, centrifuge at 250 g for 10 min and then discard the supernatant.

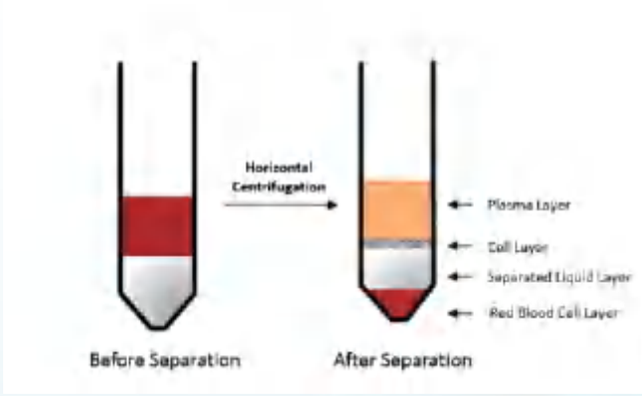
06 Repeat step 5 once.

07 Resuspend the cells with cell staining buffer for later use.

Precautions

- The temperature of Ficoll is very important. Too high or too low temperature will affect the separation effect. The optimal temperature is 20~25°C
- The blood sample should preferably be freshly anticoagulated (within 2 hours after blood collection). Freezing and refrigeration should be avoided.
- Directly freeze the sorted cell samples. Induce and detect when needed. The experiment effect is nearly same as that of direct induction (the time should be kept within 1 week).
- For intracellular factors detection, samples that cannot be detected in time after induction are recommended to be stored frozen, and the detection effect is best within 3 days. It is recommended to use 90% FBS + 10% DMSO as a freezing medium. Resuspend the cells in the freezing medium and transfer them to a cryopreservation tube. After 24 hours of cryopreservation and retesting, there is no significant difference in the results. After 3 days of cryopreservation, the expression of CD3 and IFN-γ decreased slightly. After 1 week of cryopreservation, the expression of CD3 and IFN-γ decreased significantly.

Picture of Ficoll after separation:



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Case 1

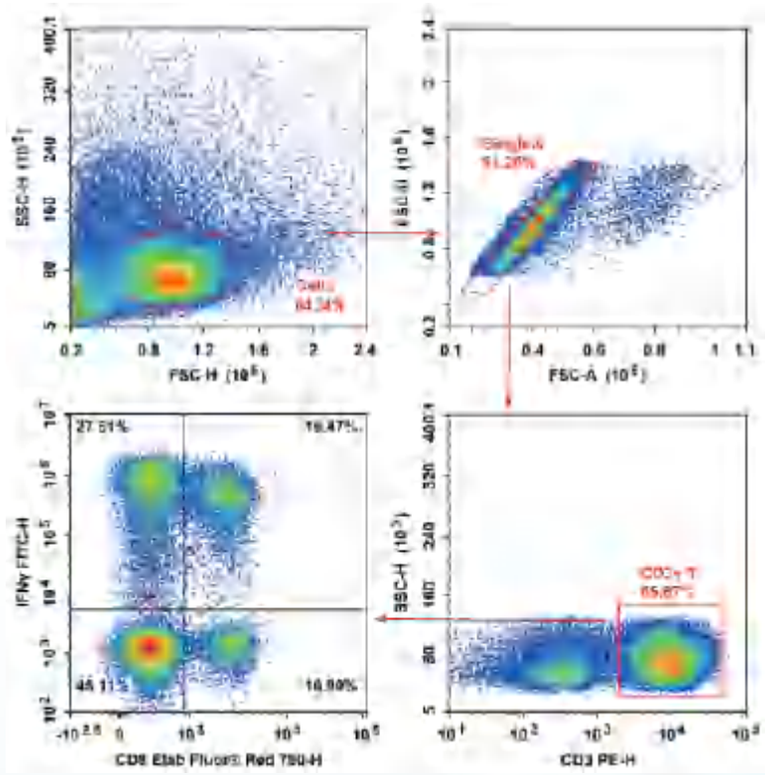
Human Peripheral Blood Th1 (3 Panels)

Panel:

Purpose	Group	Antibody Collocation
Adjust the voltage	Blank	--
Adjust compensation	Single Positive	CD3-PE
		CD8a-Elab Fluor® Red 780
		IFN-γ-FITC
Auxiliary gating	FMO and Isotype Control	CD3-PE, CD8a-Elab Fluor® Red 780, FITC Mouse IgG1, κ Isotype Control
Test	Full Panel	CD3-PE, CD8a-Elab Fluor® Red 780, IFN-γ-FITC

Information of Flow Cytometry Antibodies:

Marker	Fluorochrome	Clone No.	Cat. No.
CD3	PE	UCHT1	E-AB-F1230D
CD8a	Elab Fluor® Red 780	OKT-8	E-AB-F1110S
IFN-γ	FITC	B27	E-AB-F1196C
Mouse IgG1, κ Isotype Control	FITC	MOPC-21	E-AB-F09792C



1.PMA stimulation can lead to CD4 expression down on the surface of human T cells. Therefore, we define CD4+ T cells by gating CD3 and CD8, and CD3+ cd8-IFN-γ+ group is Th1 cells.

2.IFN-γ isotype control is necessary, since generally this marker abundance is not high.

3.The Fixation/Permeabilization buffer may damage cells, so it is recommended to re-suspend with 100 μL cell staining buffer after centrifugation, and then add the Permeabilization buffer to reduce cell damage.

Human Peripheral Blood Single Cell Suspension Preparation Process

Human peripheral blood single cell suspension preparation process

01 Collect human peripheral blood samples in anticoagulant tubes.

02 Add 100 µL of fresh blood and 2 mL of 1× Red Blood Cell Lysis Buffer to the centrifuge tube, mix and lyse at 4°C for 10 min.

03 Centrifuge at 300 g for 5 min (centrifuge immediately after lysis to prevent cells damage), discard the supernatant.

04 Wash with PBS once.

05 Add 100 µL of cell saining buffer to resuspend the cells, and directly perform subsequent flow-cytometry antibody staining experiments without counting. Add 1 Test flow-cytometry antibody to single cell suspension prepared from 100 µL of fresh blood sample and mix well to conduct experiment.

Precautions

- There are two types of anticoagulant tubes for blood collection, heparin and EDTA. If the experiment is lysed red blood cells, staining the surface targets, both tubes can be used; if the experiment is to detect cytokines, heparin anticoagulation tube must be used.
- It is recommended to use 10× ACK Lysis Buffer (E-CK-A105) as the lysate without fixative.
- The 10× ACK Lysis Buffer needs to be diluted with pure water to 1× and presently dilute it before use. It is recommended to temporarily store it at 4°C.
- For detection of routine indicators in human peripheral blood, samples stored overnight can be generally used. However, for indicators with relatively low expression levels, it is recommended to use fresh blood.
- After the step of lysis, it is recommended to remove the residual liquid with a pipette gently. If it is poured directly, there will be residual lysate in the centrifuge tube. When 100 µL of PBS is subsequently added to resuspend the cells, the lysate may not be fully diluted, resulting in excessive lysis.
- The cell pellet can be resuspended by pipetting gently, or by vortexing at a low speed.
- Human blood samples are recommended to be stained with CD45 for the subsequent data analysis.
- Human blood samples, it is recommended to set a single-stained tube that stains CD45 only and run the machine at low speed to set the threshold, observe the number of cells, and circle the lymphocyte population.
- Human peripheral blood mainly contains T lymphocytes, and the amount of B lymphocytes is relatively small. If a patient's blood sample is used, the amount of B lymphocytes may be lower. It is not recommended to use overnight samples to detect B lymphocytes, which may lead to low detection signals.

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Case 2

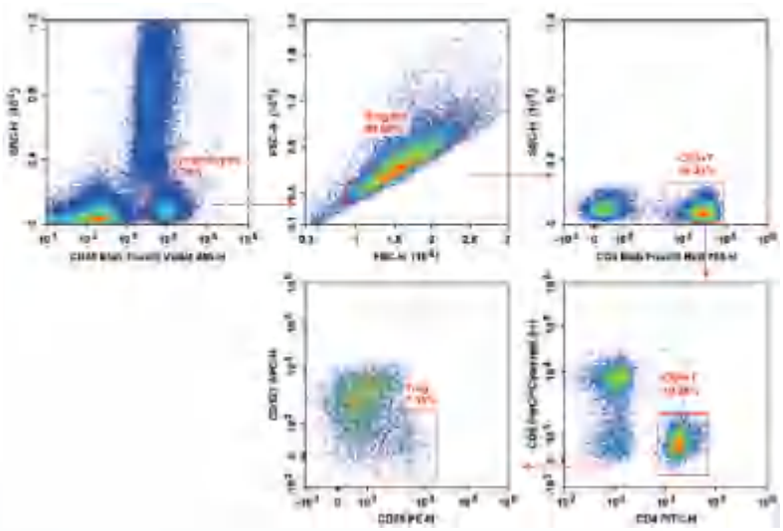
Human Peripheral Blood Treg (6 Panels)

Panel:

Purpose	Group	Antibody Collocation
Adjust the voltage	Blank	--
Adjust compensation	Single Positive	CD45-Elab Fluor® Violet 450
		CD3-Elab Fluor® Red 780
		CD4-FITC
		CD8a-PerCP/Cyanine5.5
		CD25-PE
		CD127-Elab Fluor® 647
Test	Full Panel	CD45-Elab Fluor® Violet 450, CD3-Elab Fluor® Red 780, CD4-FITC, CD8a-PerCP/Cyanine5.5, CD25-PE, CD127-Elab Fluor® 647

Information of Flow Cytometry Antibodies:

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



1. The traditional method of Foxp3 detection for Treg identification is relatively complicated, and the procedure of fixing broken membrane requires high requirements for experimenters. Currently, CD127 is used to detect human Treg.

2. The lymphocyte can be gated directly through CD45 and SSC. The cell population of CD4+CD25+CD127-/low is Treg cells, and Treg cells account for 3%-10% of lymphocytes in normal human peripheral blood.

3. This scheme needs to set single positive tubes for compensation adjustment.

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Mouse Peripheral Blood Single Cell Suspension Preparation Process and Precautions

Preparation of mouse peripheral blood single cell suspension

01 Collect peripheral blood samples from C57 mice in anticoagulant tubes.

02 Add 100 µL of fresh blood to the centrifuge tube, then add the Flow Cytometry Antibodies corresponding, mix well, and incubate at 4°C for 30 minutes in the dark.

03 Add 2 mL of 1× ACK Lysis Buffer, mix well, and lyse at 4°C for 5 min.

04 Centrifuge at 300 g for 5 min (centrifuge immediately after lysis to prevent damage to cells for too long), discard the supernatant.

05 Wash the cells once with PBS.

06 Add 200 µL of cell staining buffer to resuspend the cells, detect.

Precautions

- It is recommended to use 10× ACK Lysis Buffer (E-CK-A105) as the lysate contains no fixative.
- The 10× ACK Lysis Buffer needs to be diluted with pure water to 1× before the experiment. It is prepared and used immediately. And it is recommended to temporarily store it at 4°C.
- For the detection of routine indicators in mouse peripheral blood, samples stored overnight can be used. However, for indicators with relatively low expression levels, it is recommended to use fresh samples for detection.
- The amount of cells in the peripheral blood of mice is relatively low. It is recommended to staining the cell first and then lysing. This method can reduce the number of washed during sample processing, and reducing the loss of cells and avoiding low content cells go undetectable.

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Case 3

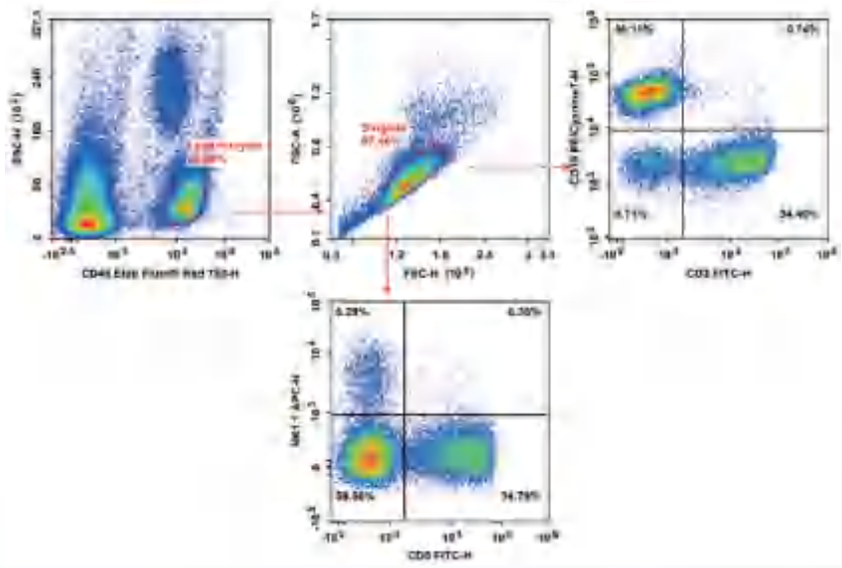
C57 Mice Peripheral Blood T/B/NK

Panel:

Purpose	Group	Antibody Collocation
Adjust the voltage	Blank	--
Adjust compensation	Single Positive	CD45-Elab Fluor® Red 780
		CD3-FITC
		CD19-PE/Cyanine7
		NK1.1-APC
Auxiliary gating	FMO and Isotype Control	CD45- Elab Fluor® Red 780, CD3-FITC、CD19-PE/Cyanine7, APC Mouse IgG2a, κ Isotype Control
Test	Full Panel	CD45-Elab Fluor® Red 780, CD3-FITC、CD19-PE/Cyanine7, NK1.1-APC

Information of Flow Cytometry Antibodies:

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



1.The lymphocyte can be gated directly through CD45 and SSC.

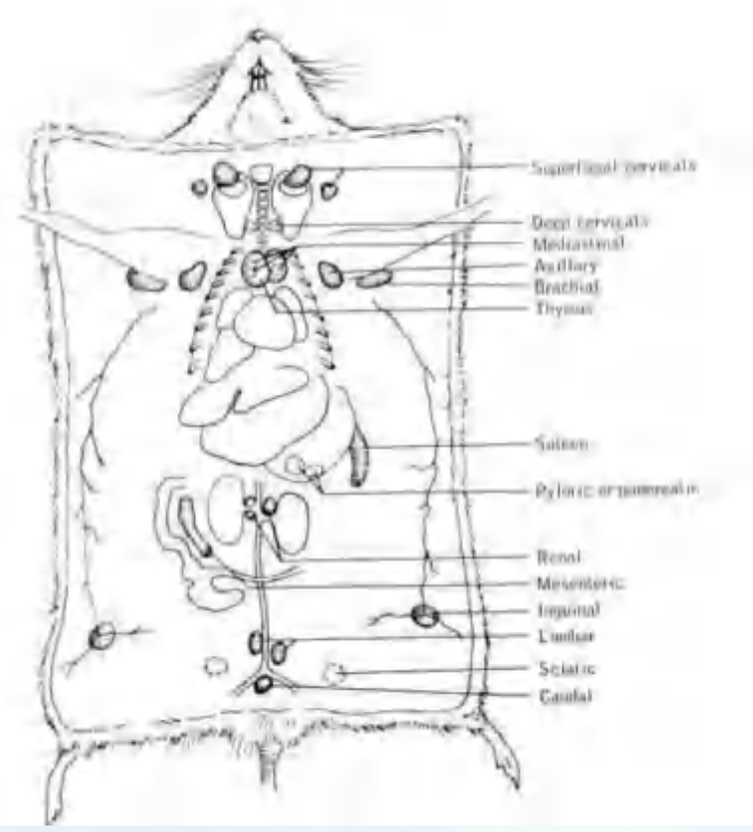
2.The CD3/ CD4/ CD8 cell population is well defined, Isotype Control is no needed.

3.The detection of NK cells, the corresponding indexes should be selected according to different mouse varieties, usually C57BL/6 mice use NK1.1, BALB/c mice use CD49b(DX5). CD3-NK1.1+/CD3-CD49b+ is NK cells.

4.The key factor in this experiment is erythrocyte lysis, otherwise the ideal experimental results will not be obtained.

Mouse Lymph Node Single Cell Suspension Preparation Process and Precautions

Precautions:



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Preparation process of mouse lymph node single cell suspension

01 Kill mouse by cervical dislocation, soak the body in 75% alcohol for 5 minutes and place it on a sterile operating table with the abdomen facing up.

02 Use scissors to cut the skin from the sternum along the midline to the lower jaw, and then cut the skin from the lower jaw to the base of the left and right ears. Hold the skin with tweezers and lift it to left and right direction, fix the skin with a needle. After the above procedure you can see a pair of large submandibular glands above the sternum. There are yellow anterior cervical lymph nodes attached to the upper borders of the left and right submandibular glands. Cut off the sternocleidomastoid muscle and muscle belly, and lift up their two severed ends. It can be seen that there is a small deep cervical lymph node on the left and right in the deep dorsal part of the left submandibular gland. Remove lymph nodes carefully with forceps and ophthalmic scissors.

03 Remove the lymph nodes and immerse them in PBS solution.

04 Aspirate the culture medium with a sterile 2.5 mL syringe, hold the lymph node with tweezers, and hold the syringe, carefully insert it into the lymph node and pipette until the lymph node cells are completely cleaned, and observe that only white connective tissue and adipose tissue remain.

05 Filter the thymocytes by pipetting through a 200-mesh sieve, collect them in a 15 mL centrifuge tube, then centrifuge at 300 g for 5 min and discard the supernatant.

06 Resuspend thymocytes with cell staining buffer, count and adjust the cell concentration to 1×10^7/mL.

Number and diameter of lymph nodes in mouse

1、Number of lymph nodes:

Mostly only one lymph node can be seen in parts of mouse body. However, 2~3 lymph nodes can be seen in the superficial neck, internal chest, stomach, pancreas and mesentery, and even as many as 4. The number of intrathoracic lymph nodes changes more often. For example, lymph nodes in the deep neck, tail or ischium, these lymph nodes are difficult to find and require more skilled anatomical techniques and careful operation. While finding lymph nodes in the superficial neck, axilla or mesentery, obtaining lymph nodes is more likely to succeed.

2、Long diameter of lymph nodes:

Most of long diameter of the lymph nodes is between 2 and 3.5 mm. The largest is mesenteric lymph node, whose long diameter is more than 10 mm, and the smallest is the deep neck, ischial and caudal lymph nodes, all of which are equal to or less than 1 mm. Generally, normal lymph nodes do not exceed 4 mm in length and diameter.

Case 4

Mouse Lymph Node T/B Cells (4 Panels)

Panel:

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

Information of Flow Cytometry Antibodies

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

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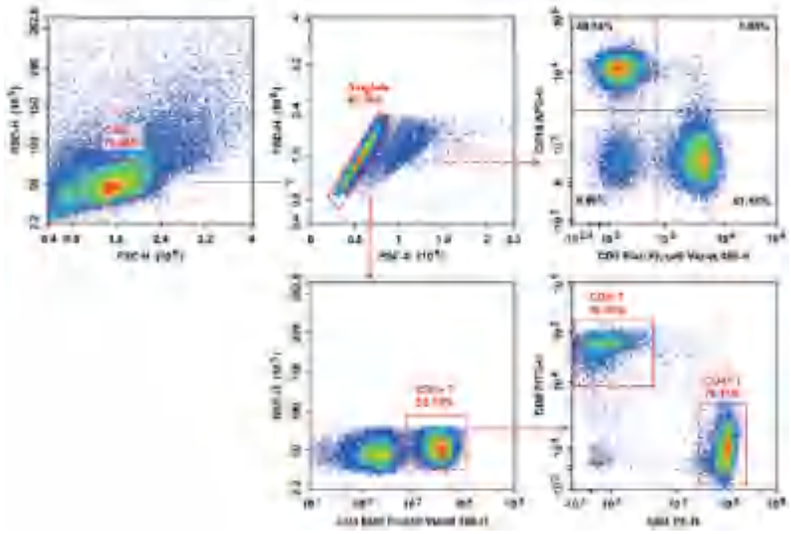

CD3/CD4/CD8/CD19 cells are easily distinguished, Single Positive, FMO and Isotype Control are unnecessary.

Mouse Spleen Single Cell Suspension Preparation Process and Precautions

Preparation process of mouse spleen single cell suspension

01 Kill mouse by cervical dislocation and soak it in 75% alcohol for 5 min, then place the mouse on a sterile operating table with the left ventral side up.

02 Cut a small incision in the middle of the left ventral side of the mouse, torn the skin open and expose the abdominal wall, a long red spleen will be visible.

03 Lift the peritoneum from the lower side of the spleen, cut it open and turn it up to expose the spleen. Lift the spleen with forceps, separate the connective tissue below the spleen with ophthalmic scissors, remove the spleen, and soak it in clean PBS solution.

Grinding method

01 Place the spleen in a 200-mesh sieve and grind gently with a tissue grinder until there are no obvious red lumps.

02 Rinse the mesh with 15 mL of PBS, collect the rinse solution in a 15 mL centrifuge tube, centrifuge at 300 g for 5 min, and discard the supernatant.

03 Add 2 mL of 1× ACK Lysis Buffer to resuspend the cells. After lysing at RT for 2~3 minutes, immediately add 10 mL of PBS. Centrifuge the solution at 300 g for 5 minutes, discard the supernatant.

04 Resuspend the spleen cells with cell staining buffer, filter the cell suspension again with a 200-mesh sieve, count and adjust the cell concentration to 1×10^7/mL.

Blowing method

01 Take a 2.5 mL sterile syringe to suck PBS, hold the spleen with tweezers and use the syringe carefully insert PBS into the spleen and pipette until the spleen cells are completely cleaned, and observe that only white connective tissue and adipose tissue remain. Pick up the remaining white tissue and rinse gently in PBS.

02 Filter the pipetted cells with a 200-mesh sieve, collect them in a 15 mL centrifuge tube, centrifuge at 300 g for 5 min, and discard the supernatant.

03 Add 2 mL of 1× red blood cell lysate to resuspend the cells, lyse at RT for 2~3 minutes and immediately add 10 mL of PBS. Centrifuge the solution at 300 g for 5 minutes, and discard the supernatant.

04 Resuspend spleen cells in cell staining buffer, count and adjust the cell concentration to 1×10^7/mL.

Precautions

- For a normal-sized spleen, about 4×10^7 cells can be obtained according to previous experience. The actual number of cells is subject to the counting result.
- Lymphocytes account for about 60%~70% of the total cells in mouse spleen after lysing red blood cells.
- For unstained spleen cell samples, a small amount (about a few tenths of a percent) of non-specific signal can be seen in the fluorescence channel.
- If there is no tissue grinding rod, the rubber pad on the tip of the pusher can also be used instead.
- If the collected spleen cells need to be further cultured, the mouse spleen should be placed on a sterile operating table. If you are just doing ordinary flow cytometry experiments, you can ignore the sterile environment.
- In the step of lysing red blood cells, the lysis time is determined by the effect of lysing during the experiment.

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Case 5

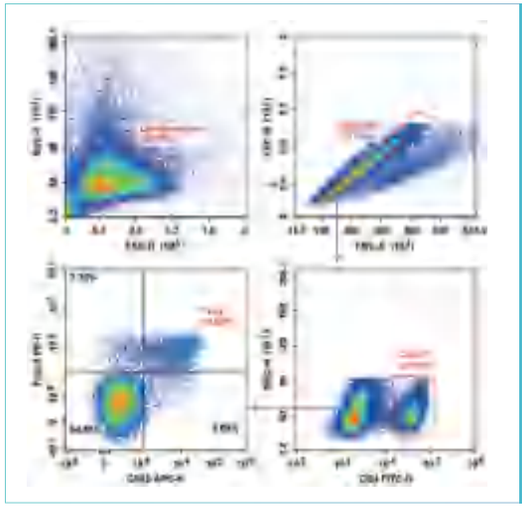
Mouse spleen Treg cells (3 Panels)

Panel:

Purpose	Group	Antibody Collocation
Adjust the voltage	Blank	--
Adjust compensation	Single Positive	CD4-FITC
		CD25-APC
		Foxp3-PE
Auxiliary gating	FMO and Isotype Control	CD4-FITC, Foxp3-PE, APC Rat IgG1, κ Isotype Control
		CD4-FITC, CD25-APC, PE Mouse IgG1, κ Isotype Control
Test	Full Panel	CD4-FITC, CD25-APC, Foxp3-PE

Information of Flow Cytometry Antibodies:

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



1.Mouse Treg market is CD4+ CD25+ Foxp3+.

2.CD4 cell group is obvious, and there is no need of isotype control. But CD25 and Foxp3 groups are not obvious, and isotype controls are needed.

3.There is fluorescence spillover, it is necessary to set single positive tubes for compensation.

4.Inappropriate use of Fixation/Permeabilization buffer may cause high background and unclear cell clustering. Please be careful.

Mouse Thymus Single Cell Suspension Preparation Process and Precautions

Preparation process of mouse thymus single cell suspension

01 Kill mouse by cervical dislocation and soak it in 75% alcohol for 5 min. Place the mouse in the sterile operating table with the ventral side facing up.

02 Cut the thoracic cavity the mouse, and the white transparent thymus can be seen. The thymus is distributed in two lobes and located in front of the two lungs, just behind the sternum.

03 Remove the thymus and soak it in PBS solution.

04 Place the thymus in a 200-mesh sieve and lightly grind it with a tissue grinder until there are no obvious lumps.

05 Rinse the mesh with 15 mL of PBS and collect the solution in a 15 mL centrifuge tube. Centrifuge the solution at 300 g for 5 min, and discard the supernatant.

06 Resuspend thymocytes in cell staining buffer, count and adjust the cell concentration to 1×10^7/mL.

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Precautions:

- Open the thoracic cavity to separate the thymus, taking care not to cut off the great vessels and break the heart.
- If necessary, inject 0.1 mL of black ink into abdominal cavity before the mouse is executed to stain the lymph nodes in the thoracic cavity, Which will facilitate lymph nodes identification and removal.
- Generally, 2×10^8 cells can be obtained from 3~6-week-old mice. Thymocytes cell number gradually decreases as mice age.

Case 6

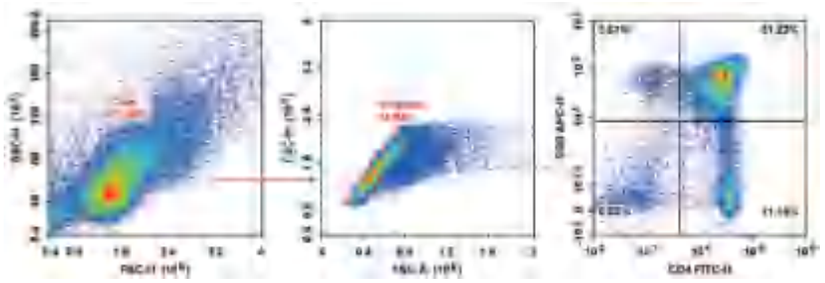
Mouse thymus cells (2 Panels)

Panel:

Purpose	Group	Antibody Collocation
Adjust the voltage	Blank	--
Auxiliary gating	FMO and Isotype Control	CD4-FITC, APC Rat IgG2a, κ Isotype Control
		CD8a-APC, FITC Rat IgG2b, κ Isotype Control
Test	Full Panel	CD4-FITC, CD8a-APC

Information of Flow Cytometry Antibodies:

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



1. According to the expression of CD4 and CD8, T cells in the thymus can be classified into double negative cells (DN cells:CD4-CD8-). double positive cells (DP cells: CD4+CD8+); single positive cell (SP cell: CD4+CD8- or CD4-CD8+).

2. The majority of T cells in the thymus co-express CD4 and CD8, so FITC and APC, two non-interfering fluorescent combinations, can reduce the complexity of data analysis.

Mouse Tumor Single Cell Suspension Preparation Process and Precautions

Mouse tumor sample preparation

01 Kill the tumor-bearing mouse by cervical dislocation and soak it in 75% alcohol for 5 min, then place the mouse on a sterile operating table.

02 Use the tweezers and the curved scissors to cut an incision of about 1 cm along the edge of the tumor. The tumor can be clearly seen attached to the subcutaneous tissue. Gently peel off the tumor.

03 Put the dissected tumor into a 100 mm Petri dish and add 5~10 mL of 1640 basal medium to the dish at RT.

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Preparation of single cell suspensions

1.Mixed enzyme digestion method

01 After all the tumors are peeled off, put the tumor into a 1.5 mL EP tube, and fully shred the tumor with curved scissors. Add 1640 basal medium while cutting and stand for a few seconds. Use a 1 mL pipette to aspirate the upper layer small particles. Continue to mince and add 1640 basal medium until all tissue sizes meet the requirements.

02 Put the tumor tissue suspension in a 50 mL centrifuge tube, add 1640 basal medium then centrifuge at 250 g for 5 min and discard the supernatant. Add 5 mL of 1640 basal medium to resuspending the cells and transfer the suspension to a petri dish.

03 Add 500 μL of 10× Triple Enzyme stock solution mixed enzyme solution to the petri dish, gently pipette until fully mixed. Transfer the solution to a 37°C carbon dioxide incubator for digestion and incubate for 1~2 h.

*10×Triple Enzyme stock solution (100 mL) (for enzymatic digestion)

Mix 1 g Collagenase IV, 100 mg Hyaluronidase and 20,000 Units DNase IV with80 mL of 1640 basal medium, then add PBS to the mixture to 100 mL. Use a 0.22 μm filter for filtration, then aliquot the filter solution to 5 mL and store it in a -20°C refrigerator. Rewarm the solution to RT before use.

04 After digestion, dilute with 1640 basal medium or PBS, then use a 200-mesh sieve to remove the remaining tissue pieces. Wash once with a large volume of 1640 basal medium or PBS buffer and obtain a single cell suspension.

05 Collect the cell suspension, centrifuge at 300 g for 5 min and discard the supernatant.

06 Resuspend the cells with cell staining buffer, count and adjust the concentration to 1×10^7/mL.

2.Grinding method

01 Prepare a 200-mesh sieve and soak it with 1640 basal medium or PBS.

02 Transfer the dissected tumor tissue to a 200-mesh cell mesh, and cut it into small particles with sterile ophthalmic scissors.

03 Use a 2.5 mL syringe plunger and grind the tissue with a soft tip in a circular motion. Take fresh 1640 medium or PBS to rinse the sieve 2~3 times.

04 Filter the obtained cell suspension with a 200-mesh sieve.

05 Collect the cell suspension, centrifuge at 300 g for 5 min and then discard the supernatant.

06 Resuspend the cells with cell staining buffer, count and adjust concentration to 1×10^7/mL.

Precautions

- 1.The tumor volume generally does not exceed 1000 mm^3 and the mass is generally between 0.6~0.8 g. If the tumor is larger, the above reaction systems will be doubled.
- 2.To minimizing cell damage during grinding, the tissue needs to be immersed in culture medium to avoid dry grinding.

Case 7

Mouse tumor infiltrating lymphocytes (4 Panels)

Panel:

Purpose	Group	Antibody Collocation
Adjust the voltage	Blank	--
Adjust compensation	Single Positive	CD45-PerCP/Cyanine5.5
		CD3-FITC
		CD4-PE
		CD8-APC
Test	Full Panel	CD45-PerCP/Cyanine5.5, CD3-FITC, CD4-PE, CD8a-APC

Information of Flow Cytometry Antibodies:

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

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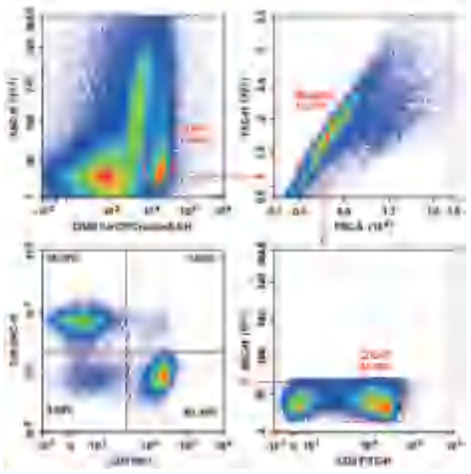
Most of the tumor tissues are tumor cells, and the proportion of lymphocytes is relatively low. Lymphocytes can be gated by CD45 and SSC.



Mouse Ascites and Single Cell Suspension Preparation Process and Precautions

Preparation process of mouse ascites and single cell suspension

01 Prepare 6% starch broth.

6% starch broth formula: 0.3 g beef extract, 1.0 g peptone, 0.5 g sodium chloride, 100 mL distilled water, mix and heat the above materials, then add 6.0 g soluble starch into the mixture. After dissolving, autoclave the solution at 121 °C for 15~20 min. The starch broth was packaged and sealed with EP tubes and stored at 4°C.

02 Inject 1 mL of 6% starch broth into abdominal cavity of mouse (do not touch intestinal tubes and internal organs) and stimulate for 60~72 h.

03 Kill mouse by cervical dislocation and soak it in 75% alcohol for 5 min.

04 Place the mouse in the dissection plate, fix the limbs and cut the skin to fully expose the peritoneum.

05 Lift the peritoneum with ophthalmic tweezers, inject 2.5 mL of pre-cooled PBS into mouse abdominal cavity with a 5 mL syringe (do not puncture the organs), and gently rub mouse abdomen for 1~2 min. Withdraw the peritoneal lavage fluid with a syringe and collect in a 15 mL centrifuge tube.

06 Repeat step 5 for 5 times, and it can be observed that the flushing fluid gradually becomes clear.

07 Centrifuge the peritoneal lavage fluid at 300 g for 5 min, and discard the supernatant.

08 Resuspend cells in Cell staining buffer or 1640 medium containing 10% fetal bovine serum (depending on the experiment to choose which liquid to use for resuspending).

09 Count the cells and adjust the cells concentration to 1×10^7/mL.

Precautions

- If there are many red blood cells in the cell pellet after centrifuging the supernatant in step 7, and the target cells are not red blood cells, add an appropriate amount of red blood cell lysate to lyse the red blood cells.
- Please pay attention to aseptic operation if you need to culture and extract peritoneal macrophages.
- When collecting ascites, inject PBS from the left side of the mouse (more intestines), and draw peritoneal lavage fluid from the right side (larger organs). Take care not to puncture the organs.
- The cells collected from the peritoneal lavage fluid of mouse are fragile. Adding cell staining buffer is beneficial to the preservation of cells, and it is recommended to detect in time on the same day.
- The majority cells in mouse peritoneal lavages are macrophages, and the detection of macrophages needs to block Fc receptors. Pure mouse CD16/32 antibody [E-AB-F0997A] can be used for blocking: add 1 μg of pure antibody to the 100 μL (Number of cells 1×10^6) cell resuspended solution, block at RT for 15 min, and directly add flow cytometry antibody for subsequent experiments.
- For detection of macrophage, the use of anthocyanin-containing flow cytometry antibodies (such as PE-Cy7) should be avoided, otherwise non-specific staining will increase. For detection of non-macrophage, blocking is also required when using anthocyanin-containing flow cytometry antibodies such as PE-Cy7.

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Case 8

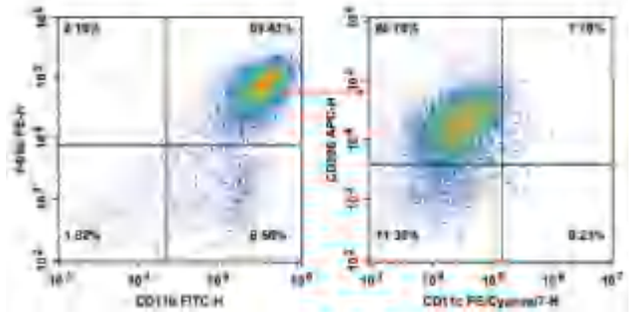
Mouse peritoneal macrophages (4 Panels)

Panel:

Purpose	Group	Antibody Collocation
Adjust the voltage	Blank	--
Adjust compensation	Single Positive	F4/80-PE
		CD11b-FITC
		CD11c-PE/Cyanine7
		CD206-APC
Auxiliary gating	FMO and Isotype Control	CD11b-FITC, CD11c-PE/Cyanine7, CD206-APC, PE Rat IgG2b, κ Isotype Control
		F4/80-PE, CD11c-PE/Cyanine7, CD206-APC, FITC Rat IgG2b, κ Isotype Control
		F4/80-PE, CD11b-FITC, CD206-APC, PE/Cyanine7 Armenian Hamster IgG Isotype Control
		F4/80-PE, CD11b-FITC, CD11c-PE/Cyanine7, APC Rat IgG2a, κ Isotype Control
Test	Full Panel	F4/80-PE, CD11b-FITC, CD11c-PE/Cyanine7, CD206-APC

Information of Flow Cytometry Antibodies:

Marker	Fluorochrome	Clone No.	Cat. No.
F4/80	PE	CI:A3-1	E-AB-F0995D
CD11b	FITC	M1/70	E-AB-F1081C
CD11c	PE/Cyanine7	N418	E-AB-F0991H
CD206	APC	C068C2	E-AB-F1135E
Rat IgG2b, κ Isotype Control	PE	LTF-2	E-AB-F09842D
Rat IgG2b, κ Isotype Control	FITC	LTF-2	E-AB-F09842C
Armenian Hamster IgG Isotype Control	PE/Cyanine7	PIP	E-AB-F09852H
Rat IgG2a, κ Isotype Control	APC	2A3	E-AB-F09832E



1. Mouse macrophages are F4/80+CD11b+.

2. M1 macrophages express CD11c and M2 macrophages express CD206; in this model, M2-type macrophages are dominant.

Mouse Bone Marrow Single Cell Suspension Preparation Process and Precautions

Preparation process of mouse bone marrow single cell suspension

01 Kill mouse by cervical dislocation and soak it in 75% alcohol for 5 min. Prepare a sterilization tray on the ultra-clean table in advance (a sterile mask or gauze soaked with alcohol can be used instead), remove and spread the mouse on the sterilization tray.

02 Use ophthalmic forceps to carefully pinch the abdominal skin between the two hip joints of the mouse, carefully cut it with ophthalmic scissors, and separate the skin of the two lower limbs. The skin was cut down at the ankle and up at the hip joint to free both hind limbs of the mouse.

03 Carefully peel off the muscle. Cut off Femurs (thigh bone) and Tibias (tibia) respectively. Cut off the cartilage at both ends and the red marrow cavity is exposed. Notice that the marrow cavity should be preserved as much as possible during this procedure.

04 Take a 1 mL sterile syringe, draw 1 mL of PBS and gently insert it into the marrow cavity. Flush the marrow cavity to obtain bone marrow. Repeat 2~3 times to flush out most of the cells. After above steps, gently pipette the cells to disperse the cell clumps.

05 Filter the rinse solution with a 200-mesh filter, collect the filtrate in a 15 mL centrifuge tube, centrifuge at 300 g for 5 min, discard the supernatant.

06 Resuspend the cells in cell staining buffer, count the cells, and adjust cell concentration to 1×10^7/mL.

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Precautions

- Bone marrow sample has obvious cell grouping, which can lyse red blood cells or not. If lyse red blood cells is needed, the procedure is as follows: add 1~2 mL (depending on the amount of cell pellet) 1× red blood cell lysate, gently blow off the cells, and stand at RT for 2 min. Add 10 mL of PBS to stop the lysis, centrifuge at 300 g for 5 min, and discard the supernatant.

Case 9

Mouse bone marrow macrophages (5 Panels)

Panel:

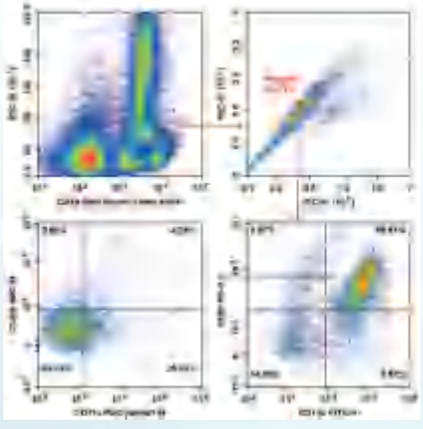
Purpose	Group	Antibody Collocation
Adjust the voltage	Blank	--
Adjust compensation	Single Positive	CD45-Elab Fluor® VIOLET 450
		F4/80-PE
		CD11b-FITC
		CD11c-PE/Cyanine7
		CD206-APC
Auxiliary gating	FMO and Isotype Control	CD11b-FITC、CD11c-PE/Cyanine7, CD206-APC, PE Rat IgG2b, κ Isotype Control
		F4/80-PE、CD11c-PE/Cyanine7, CD206-APC, FITC Rat IgG2b, κ Isotype Control
		F4/80-PE, CD11b-FITC, CD206-APC, PE/Cyanine7 Armenian Hamster IgG Isotype Control
		F4/80-PE、CD11b-FITC, CD11c-PE/Cyanine7, APC Rat IgG2a, κ Isotype Control
Test	Full Panel	CD45-Elab Fluor® VIOLET 450, F4/80-PE, CD11b-FITC, CD11c-PE/Cyanine7, CD206-APC

Information of Flow Cytometry Antibodies:

Marker	Fluorochrome	Clone No.	Cat. No.
CD45	Elab Fluor® Violet 450	30-F11	E-AB-F1136Q
F4/80	PE	CI:A3-1	E-AB-F0995D
CD11b	FITC	M1/70	E-AB-F1081C
CD11c	PE/Cyanine7	N418	E-AB-F0991H
CD206	APC	C068C2	E-AB-F1135E
Rat IgG2b, κ Isotype Control	PE	LTF-2	E-AB-F09842D
Rat IgG2b, κ Isotype Control	FITC	LTF-2	E-AB-F09842C
Armenian Hamster IgG Isotype Control	PE/Cyanine7	PIP	E-AB-F09852H
Rat IgG2a, κ Isotype Control	APC	2A3	E-AB-F09832E

1. Mouse macrophages are F4/80+CD11b+.

2. M1 macrophages express CD11c and M2 macrophages express CD206. Mouse bone marrow macrophages mainly are M1-type macrophages, the proportion of M2-type macrophages are extremely low.



Flow Cytometry Staining Protocol

Cell Surface Targets Staining for Flow Cytometry

Single cell suspension staining process

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

02 After counting the suspension with a hemocytometer or other instruments, adjust the cell concentration to about 1×10^7/mL.

03 Add 100 µL cell suspension to each tube according to the experiment design.

04 Block Fc receptor(optional), Block Fc receptors may reduce nonspecific immunofluorescent staining.

※For Mouse cells: purified Anti-Mouse CD16/CD32[E-AB-F0997A] antibody specific for FcγR III/II can be used to block nonspecific staining of antibodies. Thus, block Fc receptors by pre-incubating cells with 0.5-1µg Anti-Mouse CD16/CD32 in 100 µL volume for 10 min at RT.

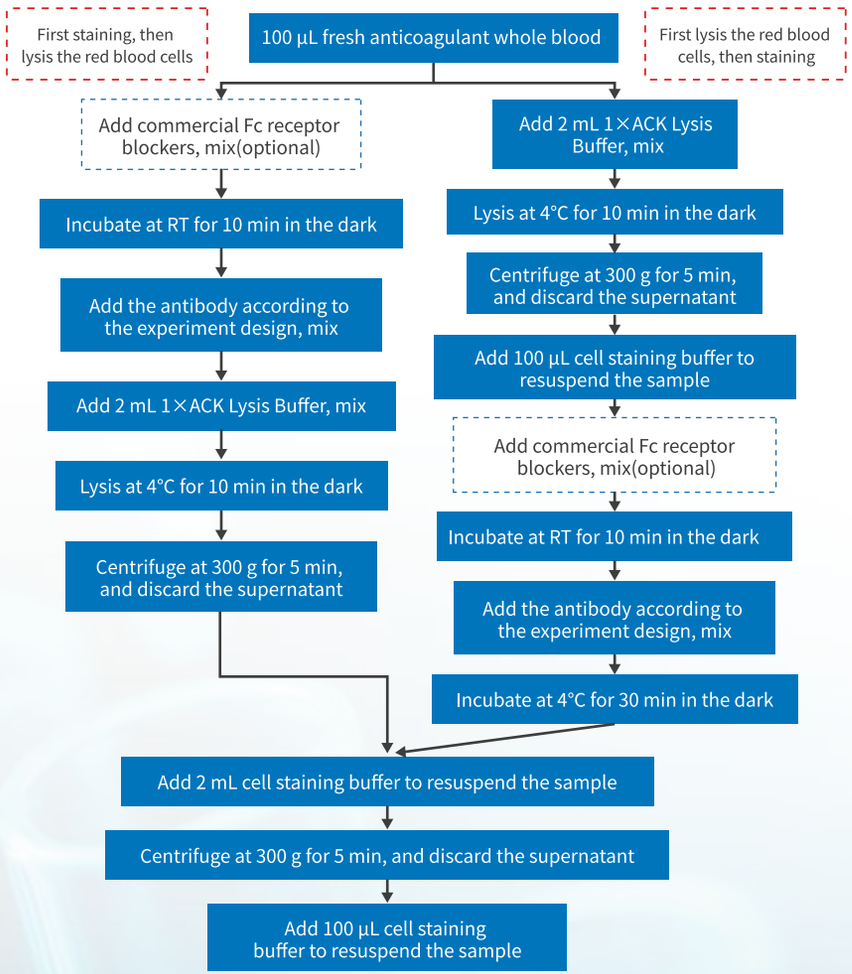
※For Human and Rat cells: Pre-incubate the cells with excess irrelevant purified Ig from the same species and same isotype as the antibodies used for immunofluorescent staining or serums from the same species as the antibody used.

05 Add the antibody according to the recommended dosage of the instructions and mix well. Incubate at 4°C for 30 min in the dark.

06 Add 2 mL cell staining buffer, centrifuge at 300 g for 5 min, and discard the supernatant.

07 Add 200 µL cell staining buffer to resuspend the sample, detect by flow cytometer.

Human whole blood staining process



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Cells Intracellular Targets Staining for Flow Cytometry

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

**If the detection index is cytokine, the cells should be stimulated and blocked the transport of secreted proteins to the extracellular.

Protocol of stimulating and blocking: (it' s recommended to use E-CK-A091)

01 Prepare the single cell suspension with complete medium (self-prepared), and adjust the cell density to 1~2×10^6/mL.

02 Add 2 µL of 500× Cell Stimulation MIX to each 1 mL of cell suspension, and incubate the cells at 37°C, 5%CO2 for 4~18 h.

03 Add 1 µL of 1000×Protein Transport Inhibitor MIX to each 1mL of cell suspension, and incubate the cells at 37°C, 5%CO2 for 5~16 h.

04 Collect cell suspension, centrifuge at 200~300×g for 5 min, discard the supernatant.

05 Resuspend the sample with cell staining buffer.

02 Count the suspension with a hemocytometer or other instruments, adjust the cell concentration to about 1 × 10^7/mL.

03 Add 100 µL cell suspension to each tube according to the experiment design.

04 (Optional) Staining Fixable Viability Dye.

05 (Optional) Block Fc receptor, Block Fc receptors may reduce nonspecific immunofluorescent staining.

For Mouse cells: purified Anti-Mouse CD16/CD32[E-AB-F0997A] antibody specific for FcγR III/II can be used to block nonspecific staining of antibodies. Thus, block Fc receptors by pre-incubating cells with 0.5-1µg Anti-Mouse CD16/CD32 in 100 µL volume for 10 min at RT.

For Human and Rat cells: Pre-incubate the cells with excess irrelevant purified Ig from the same species and same isotype as the antibodies used for immunofluorescent staining or serums from the same species as the antibody used.

06 Add corresponding antibody to each sample tube according to the specification and experiment design. Mix, incubate at 4°C for 30 min in the dark.

07 Add 1 mL cell staining buffer to each tube, centrifuge at 300×g for 5 min, and discard the supernatant.

08 Add 100 µL cell staining buffer to resuspend the sample. Add 100 µL 1× Fixation Buffer to each tube, mix gently. Incubate at RT for 30~60 min in the dark.

09 Add 0.5 mL 1× Permeabilization Working Solution to each tube, centrifuge at 600×g for 5 min, and discard the supernatant.

10 Add 100 µL 1× Permeabilization Working Solution to each tube, resuspend the sample. And add corresponding intracellular detection antibody according to the recommended dosage and the experimental design. Incubate at RT for 30 min in the dark.

11 Add 2 mL cell staining buffer and centrifuge at 600×g for 5 min, discard the supernatant.

12 Add 200 µL cell staining buffer to resuspend the sample, detect by flow cytometer.

Cells Intranuclear Targets Staining for Flow Cytometry

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

02 Count the suspension with a hemocytometer or other instruments, adjust the cell concentration to about 1 × 10^7/mL.

03 Add 100 µL cell suspension to each tube according to the experiment design.

04 (Optional)Staining Fixable Viability Dye.

05 (Optional) Block Fc receptor, Block Fc receptors may reduce nonspecific immunofluorescent staining.

※For Mouse cells: purified Anti-Mouse CD16/CD32[E-AB-F0997A] antibody specific for FcγR III/II can be used to block nonspecific staining of antibodies. Thus, block Fc receptors by pre-incubating cells with 0.5-1µg Anti-Mouse CD16/CD32 in 100 µL volume for 10 min at RT.

*For Human and Rat cells: Pre-incubate the cells with excess irrelevant purified Ig from the same species and same isotype as the antibodies used for immunofluorescent staining or serums from the same species as the antibody used.

06 Add corresponding antibody to each sample tube according to the specification and experiment design. Mix, incubate at 4°C for 30 min in the dark.

07 Add 1 mL cell staining buffer to each tube, centrifuge at 300×g for 5 min, and discard the supernatant.

08 Prepare 1 × Fixation and Permeabilization Buffer (E-CK-A108):

※1× Fixation Working Solution：dilute Fixation Concentrate (4×) with Fixation Dilution Solution to 1× Fixation Working Solution.

※1× Permeabilization Working Solution：Dilute Permeabilization Buffer (10×) with DI H2O to 1×Permeabilization Working Solution.

09 Add 1 mL of 1×Fixation Working Solution to each tube and mix, incubate the cells at 4°C for 30 min, then centrifuge at 600×g for 5 min and discard the supernatant.

10 Add 2 mL of 1×Permeabilization Working Solution to each tube and mix, centrifuge at 600×g for 5 min and discard the supernatant.

11 Repeat Step 10.

12 Resuspend the cells with 100 µL of 1×Permeabilization Working Solution. And add corresponding antibody according to the recommended dosage and the experimental design. Incubate at RT for 30 min in the dark.

13 Add 2 mL of 1×Permeabilization Working Solution to each tube and centrifuge at 600×g for 5 min at RT. Discard the supernatant.

14 Add 200 µL cell staining buffer to resuspend the sample, detect by flow cytometer.

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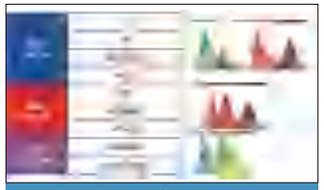
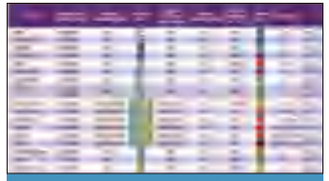
Elabscience® Featured Services

Color Schemes Services

Elabscience® provides customers with professional and free color schemes services. You only need to send your makers (logical relationship or reference, expression level) and basic information of flow cytometer (laser,-detection channel, filter information) to our technical support, and we will provide professional and free color schemes services based on your experiment purpose.



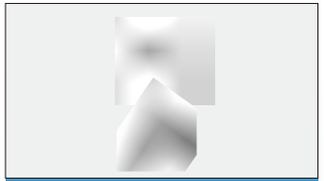
Selecte alternative fluorescent markers



Fluorescence wavelength information

Cell	Marker	Density
Iymphocyte	CD3	High
	CD4	High
	CD8	Medium
	CD19	Medium
	CD132	Low

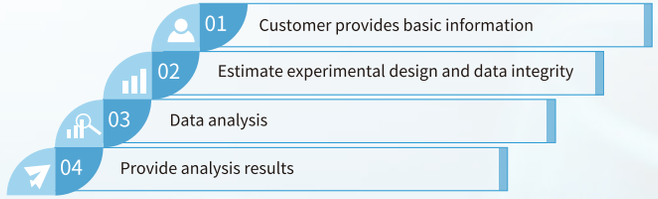
Query the strength of marker expression



Balance antigen density and fluorescence brightness

Data Analysis Services

You can also provide the original data of experimental results and logical relationship of markers to technical support. We can provide professional data analysis services for you.



Data analysis process

Elabsience® data analysis result