Mouse Peripheral Blood Single Cell Suspension Preparation Process and Precautions

Preparation of mouse peripheral blood single cell suspension

1) Collect peripheral blood samples from C57 mice in anticoagulant tubes.

2) Add 100 μL of fresh blood to the centrifuge tube, then add the flow-through antibody corresponding to 1 Test, mix well, and incubate at 4°C for 30 minutes in the dark.

3) Add 2 mL of 1× red blood cell lysis solution, mix well, and lyse at 4°C for 5 min.

4) Centrifuge at 300 g for 5 min (centrifuge immediately after lysis to prevent damage to cells for too long), discard the supernatant, and obtain a white cell pellet.

5) Wash once with PBS.

6) Add 200 μL of cell staining buffer to resuspend the cells, and use flow cytometry for detection and analysis.

Mouse Ascites and Single Cell Suspension Preparation Process and Precautions

Preparation process of mouse ascites and single cell suspension

1) Prepare 6% starch broth.

Preparation method: 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 were packaged and sealed with EP tubes and stored at 4°C.

2) 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.

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

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

5) 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.

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

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

8) Resuspend cells in Cell staining buffer or 1640 medium containing 10% fetal bovine serum.

9) Count the cells and adjust the cell concentration to 1×107/mL.

Mouse Bone Marrow Single Cell Suspension Preparation Process and Precautions

Preparation process of mouse bone marrow single cell suspension

1) 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.

2) Remove the hindlimb bones of mouse in a sterile environment. 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.

3) Carefully peel off the muscle (The white tough tissue at both ends of the muscle is tendon. Muscle is mainly connected to the joint by the tendon and can be separated along the tendon). Cut off Femurs (femur 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.

4) 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.

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

6) Resuspend the cells in cell staining buffer, count the cells, and adjust cell concentration to 1×107/mL.

Mouse Lymph Node Single Cell Suspension Preparation Process and Precautions

Preparation process of mouse lymph node single cell suspension

1) 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.

2) 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 small ophthalmic scissors.

3) Remove the lymph nodes and immerse them in clean PBS solution.

4) Aspirate the culture medium with a sterile 2.5 mL syringe, hold the lymph node with tweezers in the left hand, and hold the syringe in the right hand, 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.

5) 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.

6) Resuspend thymocytes in cell staining buffer, count the cells and adjust the cell concentration to 1×107/mL.

Mouse Spleen Single Cell Suspension Preparation Process and Precautions

Preparation process of mouse spleen single cell suspension

a) Grinding method

1) 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.

2) 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.

3) 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.

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

5) 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.

6) Add 2 mL of 1× erythrocyte lysate to resuspend the cells. After lysing at room temperature for 2~3 minutes, immediately add 10 mL of PBS. Centrifuge the solution at 300 g for 5 minutes, discard the supernatant.

7) Resuspend the spleen cells with cell staining buffer, filter the cell suspension again with a 200-mesh sieve and count, and adjust the cell concentration to 1×107/mL.

b) Blowing method

1) 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.

2) 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.

3) 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.

4) Take a 2.5 mL sterile syringe to suck PBS, hold the spleen with tweezers in the left hand and the syringe in the right hand, 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 with tweezers and rinse gently in PBS.

5) 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.

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

7) Resuspend spleen cells in cell staining buffer, count, and adjust the cell concentration to 1×107/mL.

Mouse Thymus Single Cell Suspension Preparation Process and Precautions

Preparation process of mouse thymus single cell suspension

1) Kill mouse by cervical dislocation and soak it in 75% alcohol for 5 min. Place the mouse in the sterile operating table with belly up.

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

3) Remove the thymus and soak it in clean PBS solution.

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

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

6) Resuspend thymocytes in cell staining buffer and adjust the cell concentration to 1×107/mL.

Mouse Tumor Single Cell Suspension Preparation Process and Precautions

Mouse tumor sample preparation

(1) 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.

(2) Hold the tweezers in the left hand and the curved scissors in the right hand, and 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 cut the junction along the edge of the tumor to peel off the tumor.

(3) Put the dissected tumor into a 100 mm Petri dish and add 5~10 mL of 1640 basal medium to the dish at room temperature.

Preparation of single cell suspensions

1. Mixed enzyme digestion method

1) 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.

2) 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 4.5 mL of 1640 basal medium to resuspending the cell pellet and transfer the suspension to a petri dish.

3) 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 water bath shaker for digestion and incubate for 1~2 h.

4) After digestion, dilute with 1640 basal medium or PBS, then use a 200-mesh sieve to remove the remaining tissue pieces until there are no tissue pieces. Wash once with 5~10 times volume of 1640 basal medium or PBS buffer and obtain a single cell suspension.

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

6) Resuspend the cells with cell staining buffer and adjust the concentration to 1×107/mL.

2. Grinding method

1) Prepare a 200-mesh disposable cell screen and soak it with 1640 basal medium or PBS for later use (soak and place it in a 6 cm cell culture dish or a 6-well plate).

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

3) Take a 2.5 mL syringe plunger and grind the tissue with a soft tip in a circular motion until there are no obvious tissue lumps on the sieve. Take fresh 1640 medium or PBS to rinse the sieve 2~3 times.

4) Filter the obtained cell suspension with a 200-mesh cell screen.

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

6) Resuspend the cells with cell staining buffer and adjust concentration to 1×107/mL.