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Ficoll,Density 1.077g/ml

Product Number: FC-200ml

Shipping and Storage

TStore at room temperature and avoid light, effective for two years. This product is strictly prohibited from being refrigerated or frozen for storage.

Component

Component	FC-200ml
Ficoll,Density 1.077g/ml	200mL

Description

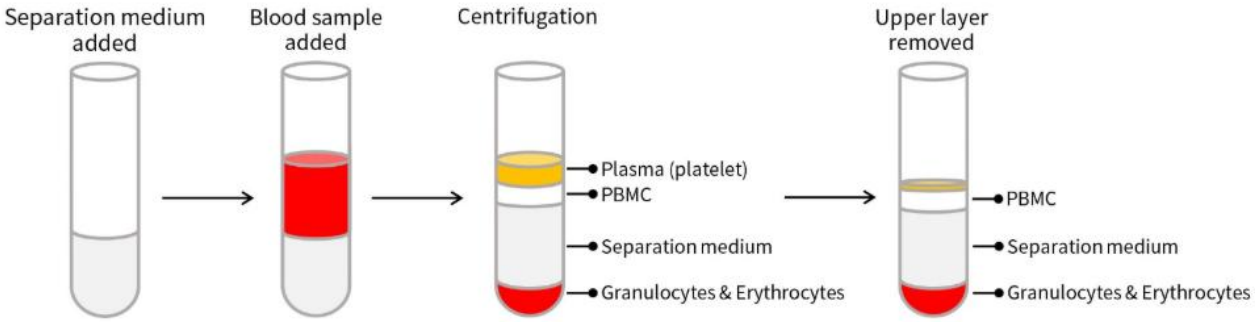
The Ficoll,Density 1.077g/ml produced by our company is a separation medium based on the principle of density gradient centrifugation, which can easily, quickly, and efficiently separate lymphocytes, monocytes, and other cells from human peripheral blood, umbilical cord blood, or bone marrow cells. It is also known as lymphocyte separation medium (LSM), lymphocyte gradient separation medium, density gradient medium, PBMC separation medium, PBMC sample density separation medium, or PBMC cell separation medium. The density of this separation solution is 1.077g/mL (20°C), which is almost isotonic with peripheral whole blood. This separation solution is almost identical in function and usage to Ficoll Paque 1.077, Ficoll Hypaque, Histoque-1077, and others.

Human peripheral blood cells include red blood cells (RBC), white blood cells (WBC), and platelets. Red blood cells are the most abundant type of blood cells in the blood and the main medium for transporting oxygen. Mature red blood cells in mammals are nucleated; White blood cells are an heterogeneous population of cells, physiologically classified into multinucleated granulocytes (including neutrophils, eosinophils, and basophils) and mononuclear cells (peripheral blood mononuclear cells, including lymphocytes and monocytes) based on their morphology, function, and origin; Platelets are small pieces of cytoplasm that break down from mature megakaryocytes in the bone marrow. They have a small volume and have coagulation and hemostatic functions.

Lymphocytes are the smallest white blood cells, accounting for approximately 20-40% of white blood cells. Lymphocytes are produced by lymphoid organs and mainly exist in the lymphatic fluid circulating in lymphatic vessels. They are cell lines with specific immune recognition functions and play an important role in immune processes such as anti-inflammatory, anti infection, and anti-tumor in the body. According to their migration, surface molecules, and functions, lymphocytes are divided into three categories: T lymphocytes, B lymphocytes, and natural killer (NK) cells. Both T lymphocytes and B lymphocytes come from hematopoietic tissues. T lymphocytes participate in cell-mediated immunity, with main immune functions including resistance to intracellular infections, anti-tumor cells, and allogeneic cells. Currently, CAR-T (Chimeric antigen receptor T cell), which has important clinical applications, is a cell-based immunotherapy based on T lymphocytes. B lymphocytes participate in humoral immunity, mainly producing antibodies, presenting antigens, and secreting cytokines to participate in immune regulation; NK cells are differentiated from lymphoid stem cells in the bone marrow and can directly exert cytotoxic effects, killing virus-infected cells, tumor cells, and allogeneic cells. Lymphocyte testing is one of the clinical routine blood tests, and abnormal lymphocytes indicate bacterial infection, viral infection, proliferative diseases of the lymphocyte system, aplastic anemia, etc. Therefore, the separation and detection of lymphocytes have important clinical significance.

The volume, morphology, and specific gravity (density) of different blood cells in human peripheral blood vary: red blood cells and granulocytes have a higher specific gravity, about 1.092; The specific gravity of mononuclear cells (lymphocytes and monocytes) is about 1.075, and platelets is about 1.030. Therefore, using a separation solution with a specific gravity between 1.075-1.090, lymphocytes and monocytes float in this separation solution due to their lower specific gravity, while red blood cells and granulocytes have a higher specific gravity and sink in this separation solution, thereby separating monocytes from peripheral blood cells. The most commonly used separation solution is a mixture of sucrose 400/Ficoll 400 and sodium dithionate with a specific gravity of approximately 1.070.

Our company's Ficoll,Density 1.077g/ml has undergone certain formula optimization and improvement, with a density of approximately 1.077g/mL at 20℃, which is almost isotonic with peripheral blood. By using this separation solution for density gradient centrifugation, the cells or liquid in the centrifuge tube are divided into four layers from top to bottom: plasma and platelets with lower density are suspended in the upper part of the separation solution, which is the first layer; The density of mononuclear cells (PBMCs, including lymphocytes and monocytes) is slightly lower than that of the separation solution, located above the interface of the separation solution. They are generally circular milky white and form the second layer, so removing the first layer can obtain PBMCs; The third layer is a transparent separation liquid layer; Red blood cells and granulocytes have a high density and settle at the bottom, forming the fourth layer.



Features

1. **This product is easy to operate, with short operation time and high separation rate**. The entire experiment only requires simple pipetting and centrifugation operations, and the separation of sample lymphocytes can be completed within about 1 hour. The extraction rate of this product for lymphocyte separation is greater than 80%. To obtain high-purity target cells, immunomagnetic beads can be used in combination for sorting. Using this reagent kit for pre separation can reduce the amount of magnetic beads used and lower costs.
2. **This product has high quality and strict quality control.** This separation solution uses pharmaceutical grade raw materials, and the formula has been optimized to be sterile with endotoxins less than 0.5EU/mL.
3. **This product has a wide range of applications.** This product is not only suitable for diluted anticoagulant blood samples, but also for undiluted anticoagulant blood samples; It can separate lymphocytes and also separate plasma of a certain purity; The isolated lymphocytes can be directly detected or cultured.
4. According to the instructions, when the sample volume is 5ml, 200ml of this product can perform approximately 40 lymphocyte separations.

Protocol

1. **Preparation before the experiment:**
   1.1. Self provided horizontal rotor centrifuge (centrifugal force ≥ 1200×g) is required. The entire gradient centrifugation requires slow rise and slow fall, and the acceleration of the centrifuge needs to be set to 1-3. When the brake is closed, it must stop naturally.
   1.2. Selection of anticoagulant: If the isolated lymphocytes are directly used for detection, sodium citrate anticoagulant can be selected; If the isolated lymphocytes need further cultivation, heparin anticoagulant should be used.
   1.3. Blood samples should be fresh anticoagulant and avoid refrigeration or freezing. The best separation effect is achieved within 2 hours of blood sample isolation; The blood sample can be separated from the body for 2-4 hours, and the separation effect is still acceptable; Blood samples are isolated for more than 4 hours, with poor separation efficiency or inability to perform separation operations.
   1.4. To ensure optimal separation efficiency, the entire experimental process requires that the sample, reagents, centrifugation, and laboratory temperature be maintained at 20±2℃.
2. Dilution of blood samples (optional):
   2.1. Dilute the sample with a buffer solution or physiological saline or culture medium that does not contain calcium or

magnesium ions.

2.2. Dilute the blood sample in a ratio of 2:1 of PBS or physiological saline.

3. Separation of lymphocytes:

3.1. Addition of separation solution and blood sample: Take a new 15ml or 50ml sterile centrifuge tube and add a certain amount of separation solution first; Then carefully tilt the tube at a 45 degree angle and slowly add the blood sample along the wall of the tube, so that the sample slides slowly along the tube wall to near the liquid level of the separation solution, avoiding the blood sample from rushing into the liquid level. At this time, the blood sample is spread flat on the liquid level of the separation solution, and attention should be paid to keeping the interface between the two liquid levels clear. Do not stir the liquid level or shake or mix well.

Note: A sterile Pap pipette (FPIP002/FPIP004/FPIP008) can be used to aspirate blood samples or subsequent plasma layers and lymphocytes; Due to the density difference between the separation liquid and blood, a distinct layered interface will be formed. If there are too many samples resulting in a longer sample addition time, it is normal for red blood cells to form clusters and sink before centrifugation; If using special peripheral blood lymphocyte isolation tubes such as SepMateTM PBMC Isolation Tube for separation, please also refer to the instructions for using the centrifuge tube.

3.2. When the sample volume is less than 3ml, add 3ml of separation solution and then operate in a 15ml centrifuge tube; When the sample volume is within the range of 3-20ml, operate the centrifuge tubes with two specifications: 15ml (step 3.3) and 50ml (step 3.4).

Note: Blood samples and separation solutions can be incubated in a 20℃ water bath for 20 minutes to ensure optimal separation temperature; The volume of separation liquid shall not be less than 3mL.

3.3. For 15mL centrifuge tubes:

3.3.1. Diluted blood sample: The recommended optimal ratio is 5ml of separation solution+4ml of diluted blood sample; The recommended optimal centrifugation conditions are 20℃, 500×g, and centrifugation for 25 minutes.

3.3.2. Undiluted blood sample: The recommended optimal ratio is 5ml separation solution+5ml blood sample; The recommended optimal centrifugation conditions are 20℃, 600×g, and centrifugation for 25 minutes.

3.4. For 50mL centrifuge tubes:

3.4.1. Diluted blood sample: The recommended optimal ratio is 20ml separation solution+20ml diluted blood sample; The recommended optimal centrifugation conditions are 20℃, 650×g, and centrifugation for 30 minutes.

3.4.2. Undiluted blood sample: The recommended optimal ratio is 20ml separation solution+20ml blood sample; The recommended optimal centrifugation conditions are 20℃, 600×g, and centrifugation for 30 minutes.

3.5. Optimization of conditions:

3.5.1. Adjust the dilution factor of the sample based on the appearance of the milky white circular layer (lymphocyte layer) after centrifugation. The milky white circular layer is dispersed, and the dilution factor of the sample should be appropriately increased; The milky white circular layer is very shallow or absent, and the dilution factor of the sample should be appropriately reduced.

3.5.2. According to the stratification position of lymphocytes after centrifugation, the centrifugal force can be adjusted appropriately. After centrifugation, lymphocytes are in the plasma layer, and the centrifugal force should be appropriately increased; After centrifugation, lymphocytes are separated in the liquid layer, and the centrifugal force should be appropriately reduced.

3.5.3. If incomplete sedimentation of red blood cells occurs, the centrifugal force can be appropriately increased.

3.5.4. If platelet contamination occurs, the centrifugal force can be appropriately reduced, and the washing frequency can be increased after separation.

3.5.5. The adjustment of centrifugal force is based on 50-100×g until the optimal separation effect is achieved. The minimum centrifugal force should not be less than 400×g, and the maximum should not be greater than 1200 ×g. The centrifugation time should be based on 20-30 minutes.

3.5.6. Regional differences, seasonal temperature differences, and differences in centrifuge performance can all affect the separation efficiency. The centrifuge conditions can be adjusted according to the actual situation. It is

recommended to maintain a constant centrifugation time and adjust the centrifugation speed when adjusting the centrifugation conditions.

3.6. Extraction of plasma layer: Carefully and slowly extract the plasma layer and transfer it to a new centrifuge tube, without touching the PBMC layer, leaving a small amount of plasma layer. The plasma layer sample can be used for plasma related testing in the future. To obtain high purity plasma, it is recommended to only draw 2/3 of the plasma layer from top to bottom, and the remaining 1/3 of the plasma may contain some PBMCs and a small amount of separated liquid components. Please refer to Figure 1 for the schematic diagram of the separation effect after centrifugation. It is divided into four layers from top to bottom, namely plasma layer (containing platelets), milky white circular layer (i.e. PBMC layer), transparent separation solution layer, and red blood cell/granulocyte layer. After centrifugation, be careful to remove the centrifuge tube and do not shake or vibrate it.

3.7. Absorption of lymphocytes: It can directly pass through the plasma layer, carefully and slowly extract the milky white circular layer (i.e. PBMC layer) and transfer it to a new centrifuge tube (this method requires high technical requirements), or carefully and slowly extract the milky white circular layer after removing the plasma layer as much as possible and transfer it to a new centrifuge tube.

Note: To avoid absorbing granulocytes at the junction of the separation solution, please do not absorb too much lymphocyte layer; If the absorbed lymphocyte layer is mixed with red blood cells, red blood cell lysis buffer (C3702) can be used to lyse the red blood cells to remove them and improve the lymphocyte separation rate.

3.8. Wash I: Add 10ml PBS or HBSS (C0218), mix well, and resuspend the cells. Centrifuge at 250 × g for 10 minutes and discard the supernatant.

3.9. Wash II: Add 5ml PBS or HBSS, mix well, and resuspend the cells. Centrifuge at 250 × g for 10 minutes and discard the supernatant. Repeat this step 2-3 times.

3.10. The obtained precipitate is the isolated lymphocytes, which can be resuspended by adding 0.5ml PBS or HBSS or adding the corresponding solution according to subsequent experimental requirements.

Note

1. Before using this product, it must be mixed upside down and absorbed under sterile conditions to avoid microbial contamination.
2. If further cultivation of isolated lymphocytes is required, attention should be paid to aseptic operation during blood collection and separation to avoid microbial contamination.
3. This product is suitable for room temperature storage and is strictly prohibited from being refrigerated or frozen. Under low temperature conditions, this product will exhibit white crystals, which will affect the separation efficiency.
4. Due to the fact that the density of this product increases at low temperatures and decreases at high temperatures, the temperature requirement for using this product is 18-22℃, and the optimal separation temperature is 20℃.
5. Different blood samples have different separation effects. Compared to peripheral blood, umbilical cord blood may have poor separation efficiency due to factors such as longer isolation time and transportation. The physical condition, medication use, disease status, and gender of the host of the blood sample may all affect the isolation effect.
6. Some plastic centrifuge tubes (such as polystyrene, PS) carry static electricity, which may cause cell wall attachment and affect separation efficiency.
7. To avoid a decrease in cell activity and improve lymphocyte separation efficiency, please dilute the blood sample with a buffer solution or physiological saline that does not contain calcium and magnesium ions.
8. Due to the differences in immune cell density among different species of animals, this product is only suitable for isolating human peripheral blood lymphocytes and is not applicable to other animals. Specific separation solutions should be used for different animal blood.
9. This product is only for scientific research by professionals and should not be used for clinical diagnosis or treatment, food or medicine, or stored in ordinary residential areas.
10. For your safety and health, please wear lab coats and disposable gloves when operating.

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Frequently asked questions

The possible problems and solutions due to differences in blood viscosity, sample dilution factors, etc. are shown in the table below.

Problem	Cause	Resolution
After centrifugation, lymphocytes exist in the plasma layer	The centrifugal force is too small or the centrifugal time is too short	Adjust centrifugal force
After centrifugation, lymphocytes exist in the transparent separation layer	Excessive centrifugal force or prolonged centrifugation time	
After centrifugation, the milky white circular layer (lymphocyte layer) disperses	The sample is not diluted or the dilution factor is too low	Adjust the dilution factor of the sample
The milky white circular layer (lymphocyte layer) after centrifugation is too shallow or invisible	The dilution factor of the sample is too high	