Human PBMC Separation Solution (P 1.077)

Cat. No: E-CK-A103 **Size: 200 mL**

Cat.	Products	Size	Storage
E-CK-A103	Human PBMC Separation Solution (P 1.077)	200 mL	RT, shading light
	Manual		One Copy

Storage

This product can be stored for one year at room temperature and protected from light.

Introduction

This product is a sterile density gradient separation solution used for separating human peripheral blood mononuclear cells (PBMC), with a density of 1.077±0.001 g/mL. Peripheral blood mononuclear cells (PBMC), mainly including lymphocytes, monocytes, phagocytes, dendritic cells and a small number of other cell types, with lymphocytes accounting for a large proportion. When the separation solution is used for density gradient centrifugation, different cells in human peripheral blood will be divided into four layers from top to bottom due to their volume, morphology, and specific gravity (density): plasma and platelets (about 1.030 g/mL) have a lower density, and they are suspended in the upper part of the separation solution, which is the first layer. The density of mononuclear cells (PBMC, about 1.075 g/mL) is a little lower than the separation solution, and it is located on top of the interface of the separation solution, which is generally ring-shaped milky white, and it is the second layer. The third layer is the transparent isolate layer. The density of erythrocytes and granulocytes have a high density (about 1.092 g/mL) and settle at the bottom, forming the fourth layer. And the PBMC can be obtained by removing the components of the top layer.

Materials Not Supplied

- **Reagents**

  RPMI-1640, L-alanyl-L-glutamine solution (200 mM), penicillin-streptomycin solution（100×）, fetal bovine serum

- **Instruments**

  Optical microscope, centrifuge

Experimental Protocol

1. Collect fresh human blood with a sodium heparin anticoagulation tubes (self-prepared);
   Note: The PBMC separation operation should be carried out within 1 hour after collecting fresh human blood. Approximately 1×10^7 PBMCs can be obtained from 10 mL of human blood. The samples, reagents and experimental environment should be kept at 20±2℃ (reagents need to be rewarmed).
2. Prepare a 15 mL centrifuge tube, add 5 mL of this reagent (to avoid liquid hanging on the wall), then slowly add 5 mL of fresh human blood along the wall of the tube (1:1 ratio, blowing and mixing before taking the

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blood). Pay attention to not to mix or shake the tube after adding (if you see obvious layering, it indicates that the blood is fresh and good), and then gently put it into a centrifuge and centrifuge it for 20 minutes at 500g.
Note: Excessive acceleration and deceleration of centrifuge may cause cell loss. It is suggested to adjust the acceleration no more than 3 and deceleration no more than 2, that is, Acc≦3, Dec≦2. After centrifugation, be careful not to shake the centrifuge tubes, and slowly put them into the ultra-clean bench for the next operation.

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 (the purpose is to improve cell yield), and transfer it to a 50 mL centrifuge tube.
4. Add RPMI-1640 basal medium (without serum) and mix fully. Centrifuge at 250× g for 5 min.
5. Discard the supernatant and keep the cell precipitate.
   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 (attention should be paid to the lysis conditions) and obtain the target cells.
6. (Optional step) Repeat washing once (steps 4-5).
7. Resuspend the cells with 1640 complete medium or add the corresponding liquid according to the next step of the experiment.

Common Problems and Solutions

Symptoms	Causes	Comments
Contamination of lymphocyte layer with red blood cells	Experimental temperature is too cold.	Bring the samples and reagents to 20±2°C before starting the experiment.
	Centrifugal speed is too low	Increase the centrifugal speed appropriately.
	Centrifugal time is too short.	Increase the centrifugal time appropriately.
Low lymphocyte viability and yield	Experimental temperature is too high.	Bring the samples and reagents to 20±2°C before starting the experiment.
	The storage time of blood is too long.	Separate fresh blood to ensure lymphocyte viability.
Contamination with granulocytes, etc	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.

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Cautions

1. This product is for research use only.
2. This product needs to be stored at room temperature and protected from light. It is easy to be infected with bacteria and needs to be operated under aseptic conditions.
3. The blood should be thoroughly mixed before adding separation solution. When adding the blood, it is important to avoid disturbing the layered liquid surface, and to ensure a good interface is formed, otherwise it may affect the separation results.
4. Centrifuge ascending speed not more than 3, descending speed not more than 2, to reduce the loss of cells caused by centrifugation.
5. Blood sample variation: individual differences and blood sample variations. For example, disease, sexuality, physical condition, etc., may result in large differences in the PBMC cells obtained from the same amount of blood or in poorer separation results.
6. Blood and separation solution in accordance with the 1:1 ratio, the separation solution is not less than 3 mL; the total liquid volume of the 15 mL centrifuge tube is not more than 10 mL, and the total liquid volume of the 50 mL centrifuge tube is not more than 40 mL.

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