Human Peripheral Blood Lymphocyte Separation Solution

Please read the datasheet carefully prior to use.
Cat. No. FB102

Version No. : Version 2.0

Storage: Stored at room temperature (15-25℃) away from light for 2 years.

Description

The volume, shape and density of mononuclear cells (mainly lymphocytes) in peripheral blood are different from other cells. The density of erythrocyte and granulocyte was high, about 1.090 g/ml, the density of platelets was 1.030 ~ 1.035 g/ml, while the density of mononuclear cells was 1.075 ~ 1.090 g/ml.This product is a sterile, nearly isotonic, density of (1.077±0.001) g/ml (20℃) glucan and meglumine solution. When using this product for density gradient centrifugation of human anticoagulant blood, the density of red blood cells and granulocyte is high, and they sink to the bottom of the separation solution; The density of peripheral blood mononuclear cells (mainly lymphocytes) was slightly lower than that of the isolation solution and located at the interface of the isolation solution, so lymphocytes with higher purity could be obtained. Ready-to-use lymphocyte isolation under sterile conditions can be used for in vitro culture and immunological testing.

Kit Contents

Component	FB102-02-V2
Human Peripheral Blood Lymphocyte Separation Solution	200 ml

Procedures

1. Balance the lymphocyte separation solution to room temperature before the experiment, and mix it upside down before opening the bottle cap. During the whole separation process, the temperature should be controlled at 15℃ -25 ℃. Too high or too low temperature will affect the density of the separation liquid, and then affect the separation effect.
2. Take fresh anticoagulant whole blood (EDTA, heparin or sodium citrate anticoagulant can be used) and dilute it with PBS or normal saline, which is equal-volume balanced to room temperature.
3. Add the separation liquid of the same volume as the undiluted whole blood into the centrifuge tube, and carefully add the diluted blood sample above the liquid level of the separation liquid to keep the interface between the two liquid levels clear. At this point, the volume ratio of undiluted whole blood, PBS (or normal saline) and the isolated solution was 1:1:1.

4. Room temperature, horizontal rotor 600-800×g centrifugation 20-30 minutes, the speed is required to slowly rise and fall. The recommended separation conditions for fresh peripheral blood within 4 hours after collection were centrifugation at 600×g for 20 minutes. The recommended separation condition for peripheral blood placed 4-8 hours after collection is centrifugation at 800×g for 30 minutes. Long time of blood sample placement will affect the separation effect.
5. After centrifugation, the centrifuge tube was divided into four layers from top to bottom, namely plasma layer, lymphocyte layer, liquid separation layer and red blood cell layer. Among them, the lymphocyte layer is a thin and dense white membrane between the plasma layer and the separation liquid layer. Carefully pipette the white film into the other centrifuge tube.
6. Dilute with 5-10 times the volume of PBS or normal saline and mix upside down. At room temperature, the horizontal rotor was centrifuged at 300×g for 10 min.
7. Discard the supernatant and repeat step (6) once.
8. The lymphocytes were resuspended with PBS or appropriate medium for reserve.

Notes

- To maintain lymphocyte activity, isolation should be performed as soon as possible after blood collection. Avoid refrigeration and freezing of blood during storage, handling and transportation.

- Care should be taken during blood collection and separation to avoid microbial contamination.

- After opening, the lymphocyte separation solution should be stored at 4℃ and used within 6 months to avoid the effect of density change due to liquid volatilization.