Human Lymphocyte Separation Medium

Cat#: 7111011/7111012

[Product Name]
Human Lymphocyte Separation Medium

[Product Parameters]
The density of mononuclear cells (lymphocytes and monocytes) in peripheral blood is between 1.075~1.090 g/ml, which is different from that of red blood cells, multinuclear leucocytes and platelets. The densities of red blood cells and granulocytes are relatively large(about 1.092 g/ml) and the density of platelets is between 1.030~1.035 g/mL. Therefore, Human Lymphocyte Separation Medium is used to generate a certain degree of density gradient, and the diluted whole blood is smoothly paved on the separation medium. After centrifugation, the red blood cells and granulocytes sink to the bottom of the tube due to their large density. Since their densities are less than or equal to the density of separation medium, lymphocytes and monocytesare found at the surface of separation medium. There may also be a small amount of cells suspended in the separation medium. By sucking the cells on the surface of separation medium, mononuclear cells can be separated from peripheral blood.

[Model & Specification]

Product Name	Cat No.	Size	Quantity
Human Lymphocyte Separation Medium	7111011	100 mL/bottle	1 bottle
	7111012	250 mL/bottle	1 bottle

[Product Parameters]

Endotoxin	Density	Osmolality
< 0.5 EU/mL	(1.077±0.001) g/mL (20℃)	(290±15) mOsmol/kg

[Storage Conditions and Shelf Life]
Kept unopened away from light at 2℃~30℃, valid for 2 years.

[Directions for Use]
1. Fresh anticoagulant (EDTA, sodium citrate or heparin and other anticoagulants are acceptable) whole blood is taken. Dilute the whole blood with an equal volume of isotonic solution (PBS or normal saline).
2. Add a certain volume of separation medium to the centrifuge tube. Carefully layer the diluted blood sample over the separation medium, keep clear interface between the two liquid surfaces. The volume ratio of separation medium, anticoagulant undiluted whole blood and isotonic solution (PBS or normal saline) is 1:1:1.
3. Centrifuge at 700 g~800 g on a swing rotor for 20~30 min at room temperature. The acceleration and deceleration are set to a slower speed (set to third gear if ten gears are available).
4. After centrifugation, the red blood cells are at bottom of the tube, the separation medium is at the middle layer, and the top layer is the plasma/tissue homogenate layer. A thin and dense white membrane found at the plasma-separation medium interface is the mononuclear cell layer (including lymphocytes and monocytes). Transfer the white membrane layer into another centrifuge tube carefully.
5. Diluted the mononuclear cell layer to a certain volume with isotonic solution (PBS, normal saline

or medium, etc.), then shake to mix well. Centrifuged at 250 g on a swing rotor for 10 min at room temperature, then remove the supernatant solution. Wash the cells once or twice.
6. Resuspend the cells with isotonic solution (PBS, normal saline or medium, etc.) and count the cells for later use.

[Applicable Instrument]
Horizontal rotor centrifuge.

[Sample Requirements]
The sample must be fresh anticoagulant blood which shall be collected by sterile operation and shall not be frozen or refrigerated in the processes of storage, handling and transportation.

[Explanation of Test Results]
Different performance of centrifuges of various brands and differences in temperature environments may have influence on the separation effect, so users can adjust the centrifugation revolution and the centrifugation time to find the best separation conditions (The specific separation conditions are determined by laboratories).

[Limitation of Test Methods]
The effect of this product is affected by such factors as storage conditions, operator experience and sample quality.

[Notes]
1. Return to room temperature before use. This product is sterile. Please open it under sterile conditions and mix it upside down before opening.
2. Isotonic solution (PBS, saline or medium) used to dilute anticoagulant whole blood should be sterile and free of calcium and magnesium ions.
3. 1:1 blood dilution can reduce the coagulation of erythrocytes and increase the amount of lymphocytes harvested.
4. For the activity of lymphocyte, blood should be separated immediately after collection. The cells harvested are peripheral blood mononuclear cells (PBMCs), including lymphocytes and monocytes.
5. Long storage of blood may cause bad result that there are more residual RBCs in the harvested lymphocytes. The time of centrifugation should be extended to improve the result.
6. If the procedures are correct but there is no layer of PBMCs found at the interfaces after centrifugation, make sure that the blood is enough and fresh, and the separation mediumis suitable for the blood of this species animal.
7. Once opened, please store the separation medium at 2℃~8℃ to avoid the change of density of the separation solution caused by liquid volatilization, which will affect the separation effect.

[Description of Product Symbol]

Product Symbol	Description	Product Symbol	Description
REF	Catalogue Number	LOT	Product Batch Code
	Date of Manufacture		Manufacturer
	Expiration Date		Temperature Limit
	Store away from light		Consult instructions for use

[Related Products]

Product Name	Cat No.	Size
RPMI-1640 Medium	6016011	500 mL/bottle
DPBS	6062011	500 mL/bottle

[Instruction Revision Date]
2024.03.22

[Company Information]
Manufacturer and after-sales service unit Name:
Shenzhen Dakewe Bio-engineering Co., Ltd.
Website:www.dakewe.com
Telephone: (86-755) 86235300
Email: RD@dakewe.com
Production Address: Room 702-703, Building No.1, Shenzhen Biomedicine Innovations Industrial Park, No.14 Jinhui Road, Kengzi Street, Pingshan District, Shenzhen
After-sales service telephone: (86-755) 86235300
Zip Code: 518122