Mouse Lymphocyte Separation Medium

Cat#: 7211011/7211012

[Product Name]
Mouse Lymphocyte Separation Medium

[Product Description]
Mouse Lymphocyte Separation Medium is a new generation of density gradient separation medium developed by Shenzhen Dakewe Bio-engineering Co., Ltd. The main component is iodixanol, with a molecular weight of 1,550. It is a completely chemically inert, non-biotoxic iodide that does not bind with any known biological function protein, does not interfere with any cell surface membrane protein, does not inhibit enzyme activity and does not interfere with antigen-antibody responses.
The lymphocyte separated by this product is with high purity, good state and high yield. The operation of Mouse Lymphocyte Separation Medium is simple, easy to learn and does not need too much experience. Our study showed that the number and quality of mouse's spleen lymphocytes did not change significantly after 1 hour of exposure to the separation medium, and the subsequent ELISPOT test results were completely consistent with those of the control group.

[Model & Specification]

Product Name	Cat No.	Size
Mouse Lymphocyte Separation Medium	7211011	100 mL/bottle
Mouse Lymphocyte Separation Medium	7211012	250 mL/bottle

[Product Parameters]

Density	Osmolality	Endotoxin
(1.081±0.001) g/mL (20 °C)	(280±15) mOsmol/kg	< 0.5 EU/mL

[Intended Use]
Separation of mouse/rat spleen lymphocytes; separation of rat/rabbit peripheral blood lymphocytes

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

[Materials Required But Not Provided]
35 mm petri dishes, the plunger of a 10 mL glass syringe, 70 μm cell strainer or nylon mesh with 200 meshes - cut into 90 mm×90 mm square (materials above are all sterile), centrifuge tubes, pipettes, tips, centrifuge with a swing-out rotor, etc.

[Directions for Use]
Separation of the spleen lymphocyte of the mouse:
1. Sacrifice the mouse by cervical dislocation; dip it in 75% ethanol.
2. Take out the spleen of the mouse at a clean bench. Operation shall be performed in an aseptic condition.
3. Add 4~5 mL Mouse Lymphocyte Separation Medium (pre-warming at room temperature and shaking before use) into a 35 mm petri dish. And grind (please refer to Figure 1 for grinding operation). The nylon mesh or cellular screen containing the spleen is dipped into the separation solution and the spleen is ground in the separation solution. Refer to Figure 1 for grinding operation.

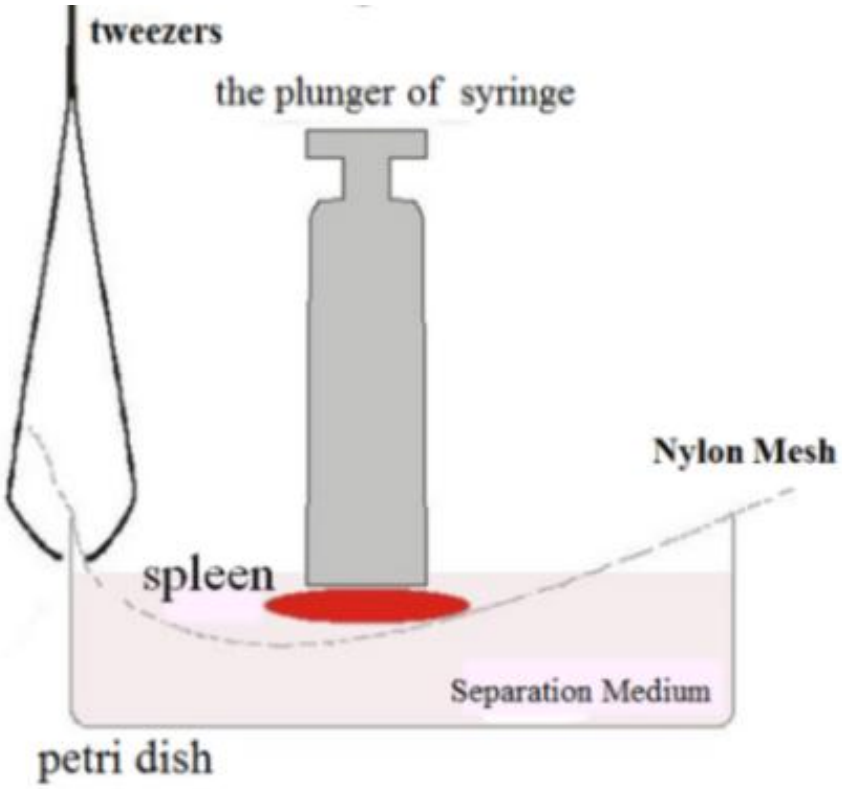

Figure 1. Process of Spleen Grinding

Special instruction - The DAKEWE method for mouse's spleen grinding:

☆ It is recommended to use nylon mesh, because it is more flexible.

☆ It is recommended to use the plunger of syringe to grind the spleen.

☆ The key is taking advantage of the rebound to control grinding, which can minimize the mechanical damage of the cells.

☆ Control the grinding strength to keep the nylon mesh suspended, so as to avoid a large number of cell death caused by direct grinding on the bottom of the petri dish.

☆ The petri dish cannot be too large, otherwise the nylon mesh cannot have effective elasticity.

☆ Clamp the nylon mesh with tweezers on one side to prevent the nylon net from sliding during grinding.

4. Transfer the cell suspension to a 15 mL centrifuge tube immediately (refer to Figure 2(A)), and cover with 500~1000 μL RPMI-1640 medium (keeping liquid level boundary clear). Please refer to Figure 2(B).

5. Centrifuge at 800 g for 30 minutes in a swing-out rotor at room temperature. The acceleration and deceleration are set to a slower speed (set to third gear if ten gears are available). The lymphocytes after centrifugation are shown in Figure 2(C).

6. Transfer the lymphocyte layer at the interface to a new centrifuge tube. Wash the cells with 10 mL RPMI-1640 medium, and centrifuge at 250 g for 10 minutes.

7. Discard the supernatant, resuspend the cells in culture medium and count.

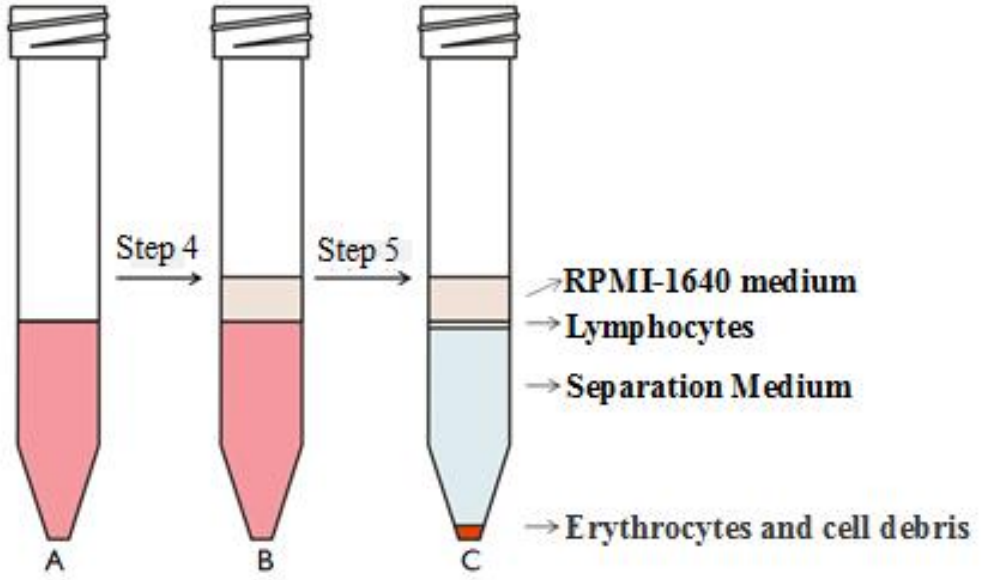

Figure 2. Process of Separating Spleen Lymphocyte with Mouse Lymphocyte Separation Medium

[Notes]

1. If the mouse is fed for a long time or the spleen is enlarged abnormally, which causes that the cell suspension after grinding shows dull red, please dilute the cells suspension with an equal volume of Mouse Lymphocyte Separation Medium and mix it well, and then proceed to step 4.
2. The separation medium is volatile so the grinding time shall be controlled within 5 minutes.
3. 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.

Separation of the spleen lymphocyte of the rat:

Because the rat's spleen is large, only a small portion of it needs to be cut for the experiment. The method of grinding and separation is exactly the same as that for the separation of mouse's spleen lymphocytes.

Separation of rat/rabbit peripheral blood lymphocytes:

1. Collect 0.5~3 mL anticoagulant blood of rat/rabbit, dilute it with an equal volume of isotonic solution (PBS or normal saline) for double dilution, and the separation effect will be better after blood dilution. The isotonic solution should not contain calcium and magnesium ions, and attention should be paid to aseptic operation.
2. Add 3 mL of Mouse Lymphocyte Separation Medium to a 15 mL centrifuge tube. Carefully layer the diluted blood over 3 mL Mouse Lymphocyte Separation Medium in a 15 mL centrifuge tube, avoid mixing the interface.
3. Centrifuge at 800 g for 15~20 minutes in a swing-out rotor at room temperature. The acceleration and deceleration are set to a slower speed (set to third gear if ten gears are available).
4. The subsequent steps are the same as the mouse's spleen lymphocyte separation operation.

[Description of Product Symbol]

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

[Related Products]

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

[Instruction Revision Date]
2024.05.30

[Company Information]
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