Mouse Peripheral Blood Lymphocyte Separation Medium

Cat. No.: M1509600 | Pack size: 200 mL | Storage: Protected from light,Room temperature

Overview

Peripheral blood mononuclear cells (PBMCs) refer to mononuclear cells in peripheral blood, including lymphocytes, monocytes, dendritic cells, and other minor cell populations (e.g., hematopoietic stem cells). PBMCs are the most commonly used cellular model for immunological function studies, such as cell proliferation, cytotoxicity, and cytokine secretion assays.

Mouse peripheral blood lymphocyte separation medium is primarily composed of Ficoll and sodium diatrizoate. It is a sterile density gradient separation medium with low endotoxin levels, specifically designed for isolating mouse peripheral blood lymphocytes. It is suitable for separating target cells from mouse blood and tissue homogenates. The separation principle is based on the density differences among blood cells (the optimal density is 1.080 for isolating mouse mononuclear cells; 1.084-1.087 for rat mononuclear cells; the density of red blood cells and granulocytes is approximately 1.092 g/mL; and the density of platelets ranges from 1.030 to 1.035 g/mL). Through centrifugation, cells of specific densities distribute according to the corresponding density gradient, thereby separating lymphocytes from mouse peripheral blood or tissues.

Key Features

- Designed for cell separation or fractionation workflows described in the product file.
- Designed for Cell fractionation workflows.
- Storage condition: Protected from light,Room temperature.

Kit Contents & Storage

Cat. No.	Component	Size	Storage
M1509600	Mouse Peripheral Blood Lymphocyte Separation Medium	200 mL	Protected from light,Room temperature

Storage: Protected from light. Room temperature

Shipped in: Normal

Materials Required But Not Supplied

Item	Recommended Specification	Purpose

Item	Recommended Specification	Purpose
Sterile pipettes, tubes, and centrifuge	Cell culture grade	Sample processing
PBS or isotonic buffer	Sterile, calcium/magnesium-free if specified	Cell washing and dilution
Appropriate separator or instrument	As required by protocol	Cell separation or downstream processing

Specifications

Attribute	Value
Application	Cell fractionation
Shipped In	Normal
Specifications & Purity	BioReagent, sterile-filtered
Stability And Storage	Store at room temperature long term (24 months). Store in the dark.
Storage	Protected from light, Room temperature

Protocol

1. Dilute the blood sample: Mix 1 volume of isotonic solution (e.g., PBS buffer) with 1-2 volumes of blood, resulting in a ratio of isotonic solution: anticoagulated blood=1:1-2.

2. To meet practical experimental needs, the recommended operational protocols based on different blood sample volumes are as follows:

Scenario A: Blood sample volume<2 mL

1. Take a 15 mL centrifuge tube and add 4 mL of separation medium.

2. Carefully pipette the diluted blood sample and layer it gently onto the surface of the separation medium. Centrifuge at 450-500×g for 20-30 min. (Note: Centrifugation parameters depend on the blood sample volume. A larger sample volume requires higher centrifugal force and longer centrifugation time. Users should optimize these parameters to achieve the best separation efficiency.)

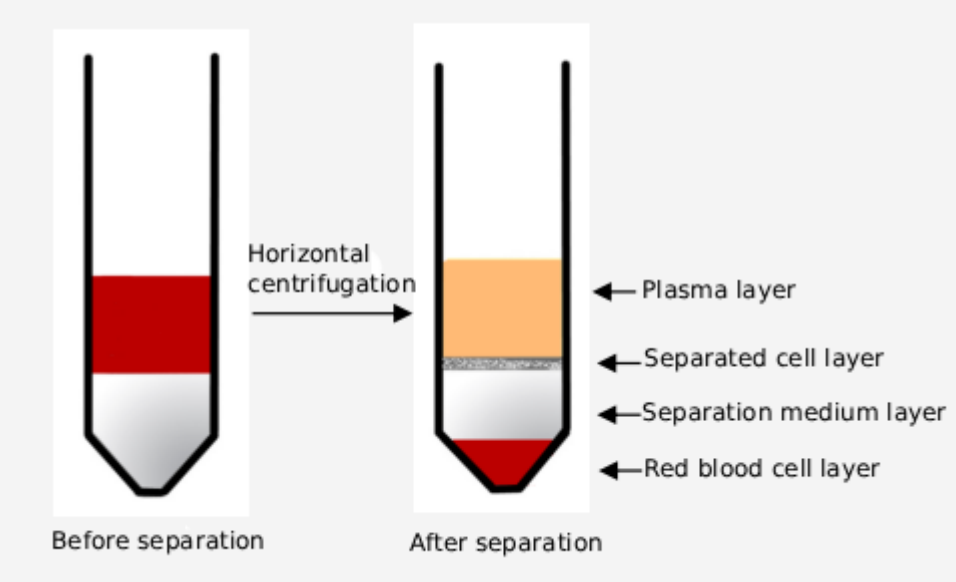
3. After centrifugation, the contents in the centrifuge tube are separated into four distinct layers from top to bottom:

Layer 1: Plasma layer

Layer 2: Opaque milky white lymphocyte layer

Layer 3: Transparent separation medium layer

Layer 4: Red blood cell layer (As illustrated in the figure below)



4. Carefully aspirate the second layer (opaque milky white lymphocyte layer) into a new 15 mL centrifuge tube. Add 10 mL of isotonic solution to the tube and resuspend the cells thoroughly.

5. Centrifuge at 250×g for 10 min.

6. Discard the supernatant.

7. Resuspend the collected cells with 5 mL of isotonic solution using a pipette.

8. Centrifuge at 250×g for 10 min.

9. Repeat steps 6, 7, and 8. After discarding the final supernatant, resuspend the cells in 1 mL of the appropriate medium required for subsequent experiments.

Scenario B: Blood sample volume=2-4 mL

1. Take a 15 mL centrifuge tube and add 5 mL of separation medium.

2. Carefully pipette the diluted blood sample and layer it gently onto the surface of the separation medium. Centrifuge at 450-650×g for 20-30 min. (Note: Centrifugation parameters depend on the blood sample volume. A larger sample volume requires higher centrifugal force and longer centrifugation time. Users should optimize these parameters to achieve the best separation efficiency. The maximum centrifugal force is recommended not to exceed 1200×g.)

3. After centrifugation, the contents in the centrifuge tube are separated into four distinct layers from top to bottom:

Layer 1: Plasma layer

Layer 2: Opaque milky white lymphocyte layer

Layer 3: Transparent separation medium layer

Layer 4: Red blood cell layer

4. Carefully aspirate the second layer (opaque milky white lymphocyte layer) into a new 15 mL centrifuge tube. Add 10 mL of

isotonic solution to the tube and resuspend the cells thoroughly.

5. Centrifuge at 250×g for 10 min.

6. Discard the supernatant.

7. Resuspend the collected cells with 5 mL of isotonic solution using a pipette.

8. Centrifuge at 250×g for 10 min.

9. Repeat steps 6, 7, and 8. After discarding the final supernatant, resuspend the cells in 1 mL of the appropriate medium required for subsequent experiments.

Product Performance Characteristics

Appearance: Opalescent or slightly opalescent aqueous injection solution.

Density: 1.081±0.001 g/mL.

Osmolality: 280-340 mOsmol/kg.

Sterility: Filtered through a 0.22 μm membrane.

This product is for research use only and is not intended for clinical diagnosis or any other purposes.

Sample Handling

Process blood, tissue, or cell suspensions as soon as possible after collection. Keep samples at the temperature specified in the protocol and avoid harsh mixing that may reduce cell recovery or viability.

Use clean, sterile buffers and tubes. Prepare a single-cell suspension or clarified sample as required before loading onto the separation medium or magnetic separation workflow.

Safety & Precautions

1. Invert and mix well before opening. This separation medium is a sterile product and is susceptible to bacterial contamination; therefore, all operations must be performed under sterile conditions. After opening under sterile conditions, store the product at 4°C, with a validity period of 6 months.
2. The separation medium should be maintained at room temperature (18°C-25°C) during use. If the ambient temperature is too low, pre-warm the medium appropriately. Centrifugation at 4°C or lower temperatures may increase red blood cell contamination in the buffy coat layer.
3. The blood sample should preferably be fresh anticoagulated blood (collected within 2 hours). To maintain lymphocyte viability, avoid freezing and refrigeration of the sample.
4. For blood dilution or cell washing, do not use buffers or culture media containing Ca^{2+} or Mg^{2+}-these ions can cause blood cell agglutination, which significantly reduces cell yield and purity.
5. Some plastic materials (e.g., polystyrene) may cause cell adhesion due to electrostatic effects, thereby affecting separation efficiency.
6. Variations in blood sample viscosity or temperature may impact separation results. Adjust centrifugation speed and duration to optimize separation conditions.
7. Aspirating an excessive amount of the lymphocyte layer or separation medium layer may lead to the inclusion of granulocytes at the interface, increasing granulocyte contamination. Aspirating an excessive amount of the plasma layer may result in contamination of lymphocytes with plasma proteins and platelets.
8. If further culture of the isolated cells is required, ensure strict sterile operation during blood collection and separation processes to avoid microbial contamination.
9. Different animal blood samples exhibit varying cell dispersion coefficients and cell charge properties in separation media of different densities. When preparing custom separation media, users should specify the required medium density, animal species, and the name of the target cells to be isolated.
10. This product is light-sensitive and should be stored at room temperature in the dark.
11. For your safety and health, wear a lab coat and disposable gloves during operation.
12. Read the current SDS before use. Wear lab coat, gloves, and eye protection. Handle reagents according to local laboratory safety and EHS procedures.
13. Use clean or sterile consumables as required by the application and avoid contamination during handling.
14. Follow the storage condition on the product label. Protect from light or freezing where specified.

Quality Control

QC Item	Method	Acceptable Range
Appearance and sterility-related condition	Visual inspection / lot COA	No leakage, turbidity outside specification, or contamination
Separation performance	Representative sample or control workflow	Cell fraction, purity, or recovery meets expected application requirements
Storage condition verification	Receipt and storage review	Stored as specified; do not freeze where indicated

Troubleshooting

Issue	Possible Causes	Corrective Action
Low cell recovery	• Sample age or condition unsuitable • Centrifugation or magnetic separation parameters not optimized • Loss of target fraction during transfer	• Use fresh samples and recommended buffers • Optimize time/speed according to sample volume • Aspirate/transfer layers carefully
Low purity	• Incorrect buffer or density condition • Disturbed interface or bead carryover • Insufficient washing	• Use specified buffer and temperature • Avoid disturbing non-target fractions • Repeat wash/separation steps as appropriate
Reduced cell viability	• Delayed processing • Improper temperature • Harsh mixing	• Process samples promptly • Maintain recommended temperature • Mix gently and avoid vigorous shaking

Recommended Applications

Cell fractionation.

Contact & Global Offices

Whether you have a technical question, need help with a quotation, or want to inquire about an order, our regional teams are ready to assist. Please contact the office for your region; for general inquiries, the North American office is the corporate primary.

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Limitations & Disclaimer

- For Research Use Only (RUO). Not for use in human or animal diagnostics, therapeutics, or in vivo applications. Not for food, cosmetic, or household use.
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