Rat Bone Marrow Lymphocyte Separation Solution

Catalog No.: RC0208

Product Description

This product is a sterile, low-endotoxin density gradient separation medium designed for isolating lymphocytes from bone marrow. The separation principle relies on cell density differences—cells are distributed along corresponding density gradients via centrifugation, thereby enabling lymphocyte isolation. It is suitable for isolating lymphocytes from bone marrow, and lymphocytes obtained under sterile conditions can be used for cell culture.

This kit ensures >80% recovery and purity of target cells. For higher-purity T cell or B cell populations, additional immunomagnetic bead-based separation is recommended. This kit can reduce magnetic bead consumption and lower costs.

Product Specifications

Item	Volume
Bone Marrow Lymphocyte Separation Medium	200ml
Homogenization Wash Buffer	200ml

Transport and Storage

- **Transport:** Room temperature
- **Storage:** Store at 15–25°C protected from light. **DO NOT refrigerate or freeze.** Storage at 4°C may cause white crystallization, compromising separation performance.
- **Handling:** All operations must be performed under sterile conditions.

Pre-Experiment Preparation

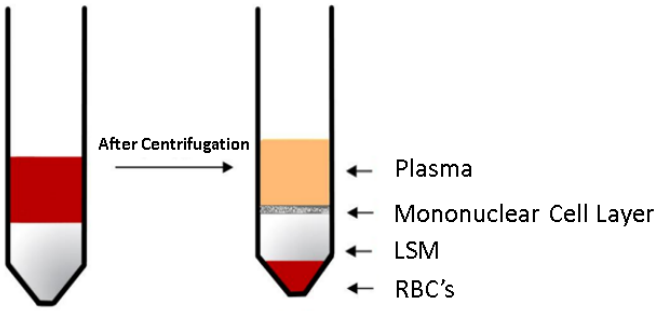
Preparation of Bone Marrow Mononuclear Cell Suspension

1. Euthanize the animal using an appropriate method. Dissect out femurs or tibias, and cut off both ends of the bone to expose the marrow cavity. Flush the marrow from the cavity using an appropriate volume of homogenization buffer (according to animal size) and a syringe.
2. Transfer the marrow or marrow suspension into a suitable centrifuge tube. Gently pipette to create a single-cell suspension, then filter through a **70 µm cell strainer**.
3. Centrifuge at **450 × g for 10 min**. Discard the supernatant.
4. Resuspend the cell pellet in PBS to a final concentration of **2×10^8–1×10^9 cells/mL** (for mice, typically resuspend marrow cells in 1 mL of PBS buffer). The cell suspension is now ready for separation.

Protocol

1. **Prepare Tube:** Add 4.0 mL of separation solution to a sterile 10 mL siliconized centrifuge tube.
2. **Layer Blood Sample:** Slowly add 2.0–4.0 mL of blood sample (or cell suspension) by pipetting **along the tube wall** onto the surface of the separation solution to form a clear interface. **Do not mix.** Place into centrifuge immediately.

3. **Centrifuge:** Spin at **500 g for 30 minutes**.
   Note: Optimize centrifugation conditions based on blood sample volume. Larger volumes require higher g-force and longer time. Optimize empirically for best results.
4. **Harvest Cells:** After centrifugation, the tube will show four distinct layers from top to bottom:
   - **Layer 1:** Plasma/diluent layer
   - **Layer 2:** White, ring-shaped lymphocyte/mononuclear cell layer *(target cells)*
   - **Layer 3:** Clear separation medium layer
   - **Layer 4:** Red blood cell layer
5. **Aspirate Plasma:** Carefully remove and discard the plasma layer. Then gently aspirate the white ring layer (Layer 2) and transfer to a new centrifuge tube.
6. **Wash Cells:** Add 5–10 mL PBS buffer to the tube containing target cells. Mix gently and centrifuge at **250–400 g for 10 minutes**. Discard supernatant.
7. **Second Wash:** Resuspend cell pellet in 5 mL PBS buffer. Centrifuge at **250–400 g for 10 minutes**. Discard supernatant.
8. **Final Resuspension:** Resuspend cells in 0.5 mL PBS buffer or appropriate medium for downstream applications.



Precautions

1. **Use appropriate consumables:** Avoid high-polymer materials (e.g., polystyrene) due to static electricity. Use static-free or low-static centrifuge tubes and non-alkali-treated glassware, as static causes cell adhesion to tube walls and alkali-treated glass surfaces become roughened, compromising separation efficiency.
2. **Avoid plasma contamination:** Aspirating excessive material above the target cell layer will introduce plasma proteins and platelets.
3. **Proper dilution is critical:** Improper dilution reduces cell yield and viability. **Diluent requirements:** Calcium/magnesium-free buffer or medium. If blood is diluted, reduce centrifugal force and time accordingly.

Troubleshooting

This separation solution's density is sensitive to temperature and atmospheric pressure. Users should adjust centrifugation parameters based on local conditions. **When optimizing, keep centrifugation time constant (20–30 min) and adjust speed.

- Adjustment increments:50–100 g
- Operating range: Minimum 400 g, maximum 1200 g

Note: Incomplete RBC sedimentation may occur due to pharmaceutical-grade raw materials meeting international standards. Gently increase centrifugal speed if necessary.