Rat Peripheral Blood Lymphocyte Separation Solution

Catalog No.: RC0202

Product Description

This product is a sterile, low-endotoxin density gradient separation medium designed for isolating lymphocytes from rat peripheral blood. The separation principle relies on density differences among blood cells, where centrifugation distributes cells according to their respective densities, thereby separating lymphocytes from peripheral blood. The lymphocytes isolated under sterile conditions are suitable for cell culture applications.

This kit ensures a target cell recovery rate and purity of **>80%**. For higher purity T cells or B cells, please combine with immunomagnetic bead sorting. This separation solution reduces magnetic bead consumption and associated costs.

Product Specifications

Volume: 200 mL

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

Optimal Separation Time

Blood must be processed within **2 hours** of collection. If this cannot be achieved, separation must be performed within **4 hours**. Separation after 4 hours will be significantly compromised.

Time Post-Collection	Separation Performance
Within 2 hours	Optimal
2–4 hours	Acceptable
4–6 hours	Decreased viability, poor separation
Over 6 hours	Very poor separation, may fail completely

Optimal Separation Conditions

- **Aseptic Technique:** Strictly follow aseptic procedures in a laminar flow hood or biosafety cabinet.
- **Temperature:** Perform procedure at 18–22°C ambient temperature; **20°C yields optimal results**. Temperature deviations may alter separation medium density and compromise performance. Allow reagents to equilibrate to recommended temperature before use (20°C in summer, 25°C in winter).

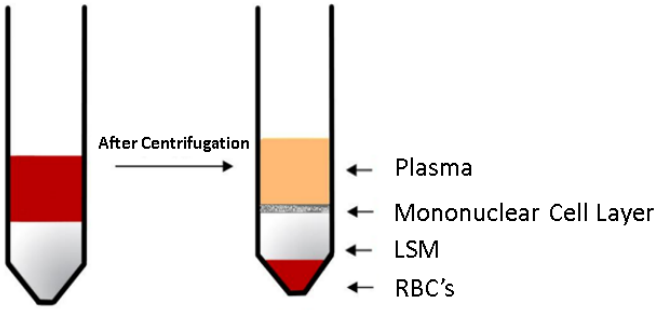
Blood Sample Dilution (If Required)

Dilution Method: Mix 1 part PBS with 2 parts blood (PBS:anticoagulated blood = 1:2).

Note: Use calcium/magnesium-free buffer or medium for blood dilution.

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

1. Issues Related to Blood Viscosity/Cell Density

Observation	Cause	Recommended Solution
Target cells in plasma/diluent layer	Insufficient g-force or centrifugation time	Increase centrifugal speed appropriately
Target cells in separation medium layer	Excessive g-force or centrifugation time	Decrease centrifugal speed appropriately
Diffuse/irregular white ring layer	Cell density too high	Adjust cell density
Shallow or absent white ring layer	Cell density too low	Adjust cell density

2. **Red Blood Cell Contamination in Target Layer**

- Transfer the RBC-contaminated cell layer to a new tube.
- Add 5–8 volumes of RBC lysis buffer based on residual RBC count. Perform multiple small-volume lysis cycles to obtain lymphocytes (refer to RC0211 RBC Lysis Buffer manual).

3. Adjustment for Local Conditions

This separation medium's density is sensitive to temperature and atmospheric pressure. Users in different regions should adjust centrifugation parameters accordingly. **When optimizing, keep centrifugation time constant (20–30 min) and adjust speed.**

- **Speed adjustment increments:** 50–100 g
- **Operating range:** Minimum 400 g, maximum 1200 g

4. Incomplete RBC Sedimentation

Due to pharmaceutical-grade raw materials meeting international standards, performance may differ slightly from domestic products, occasionally causing incomplete RBC sedimentation. **Gently increase centrifugal speed** if this occurs.