Rat Spleen Lymphocyte Separation Solution

Catalog No.: RC0204

Product Description

This product is a sterile, low-endotoxin density gradient separation medium designed for isolating lymphocytes from rat spleen tissue. The separation principle relies on density differences among cells, where centrifugation distributes cells according to their respective densities, thereby separating lymphocytes from spleen tissue. 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, combine with immunomagnetic bead sorting. This separation solution reduces magnetic bead consumption and associated costs.

Product Specifications

Item	Volume
Spleen Lymphocyte Separation Solution	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 Single-Cell Suspension from Spleen Tissue

1. **Mince Tissue:** Weigh the tissue and use sterile ophthalmic scissors to cut the spleen into small fragments.
2. **Mechanical Dissociation:** Place tissue fragments on a 70 µm cell strainer and grind repeatedly with a pestle while adding Homogenization Wash Buffer (approximately 5–8 mL per 0.1 g tissue) to flush single cells through the mesh into a centrifuge tube.

Note: Cells should form a single-cell suspension that passes through the strainer, not be squeezed as tissue clumps. The goal is to generate single cells, not cell aggregates or tissue debris, as single cells separate more efficiently.

3. **Initial Wash:** Discard the strainer. Centrifuge the tissue homogenate at 300–400 g for 10 min and discard the supernatant.
4. **Cell Count Adjustment:** Resuspend cells in PBS buffer to a concentration of 2×10^8–1×10^9 cells/mL. (For example, resuspend 0.1 g spleen tissue in 0.5–1 mL PBS buffer.)

Important Notes:

a) When processing multiple animal spleens, process each sample individually; do not combine tissues for simultaneous grinding.

b) For mouse or rat spleens, resuspend in approximately 0.5 mL or 1.0 mL PBS buffer,

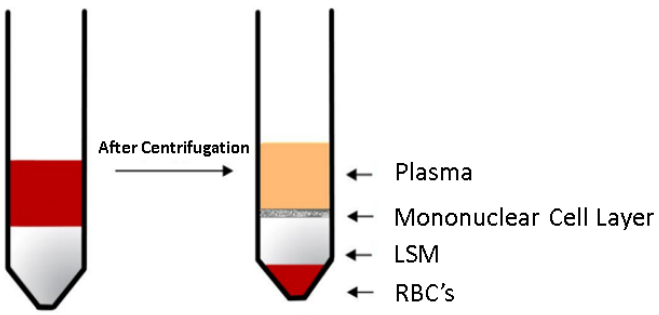
respectively, to achieve 2×10^8–1×10^9 cells/mL.

c) If the cell suspension remains viscous after washing

5. **Prepare fresh wash buffer:** Mix 9 parts Homogenization Wash Buffer with 1 part trypsin/EDTA solution.
6. **Pre-treat centrifuge tube:** Add 100–500 µL fetal bovine serum or species-matched serum to the tube before grinding to neutralize trypsin/EDTA digestion.
7. Proceed with steps 2–4 above.

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.