Isolating PBMCs From LRS Chambers Using RBC Lysis Only

Blood Centers of America
https://bcaadvancedtherapies.com/lrs-chambers/

Isolate PBMCs from the RBC fraction of LRS chambers in 30-40 minutes using this simple RBC lysis protocol.

Supplies and Equipment Needed

- LRS chamber
- 70% isopropyl alcohol
- Kim Wipes
- 15 mL conical tube
- 50 mL conical tube
- Test tube holder for each tube size
- Sterile or sterilized scissors
- 18G blunt-end needle (optional)
- 5 mL or 10 mL syringe (optional)
- Plastic transfer pipettes
- 5 mL serological pipet
- Phosphate Buffered Saline (PBS)
- PBS buffer with 2% human serum or fetal bovine serum
- Isotonic RBC lysis buffer containing Ammonium Chloride, Potassium Bicarbonate, and EDTA ("ACK Lysis Buffer"). Make your own or buy this one.
- Tabletop centrifuge capable of holding 15 mL and 50 mL conical tubes
- Biological Safety Cabinet if keeping cells sterile

*Technical Questions: If you have any technical questions about the product, please **click here** to submit your inquiry by email and we will have someone contact you.*

< see next page for the protocol >

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Protocol

*If sterility is required, practice sterile techniques, use sterilized supplies and equipment, and perform all open system steps inside a biological safety cabinet.

1. Wipe LRS chamber with a Kim Wipe soaked in 70% isopropyl alcohol.
2. Using sterile scissors, clip the tubing on the wide end of the LRS chamber and place over an open 15 mL conical tube.
3. Clip the top tubing with the scissors, leaving about ½ inch of tubing attached to the chamber.
4. Let the contents of the LRS chamber drip into the 15 mL tube.
    a. If the dripping stops before the chamber is empty, unclog the bottom tubing by gently pushing air through the top tubing using a blunt-end needle and syringe.
    b. Do not add any buffer to the chamber.
5. Cap the tube and centrifuge at 1200 RPM for 10 min at room temperature.
6. Remove the tube from the centrifuge, taking care not to disturb the separated layers of plasma, WBCs, and RBCs
7. Using a plastic transfer pipette, remove the plasma and white blood cell layers and transfer into a 50 mL conical tube. Also transfer ~1 mL of RBCs from near the WBC layer.
8. To the harvested cells, add 5 mL of ACK buffer.
    a. Adjust the volume of buffer as needed.
9. Cap the tube and swirl for 1 minute.
10. Add PBS buffer to the top of the tube and centrifuge at 1200 RPM for 10 min at room temperature.
11. Remove the wash supernatant, taking care not to disturb the cell pellet.
12. If needed, repeat steps 8-11 above, adjusting the volume and incubation time as needed to complete the lysis of remaining RBCs
13. Resuspend cells in PBS buffer containing human or fetal bovine serum
14. Continue directly to downstream applications.

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