Protocol – Isolation of PBMCs Using a Density Gradient Medium

Materials

LEUKOMAX® leukopak
Density gradient medium
HBSS wash buffer
ACK lysis buffer (optional)
Detergent disinfectant
70% isopropanol
50 mL sterile conical tubes
Large sterile container for initial LEUKOMAX® leukopak contents

Equipment

Biological Safety Cabinet (BSC)
Serological pipette and pipettors
Centrifuge
Scissors

Notes:

Wear appropriate personal protective equipment (PPE) when performing this protocol
Handle biological specimens with precaution
Ensure centrifuges have been properly balanced before starting to limit processing time
vapor phase" of a liquid nitrogen tank.

Protocol:

1. Record the initial product volume from the Certificate of Analysis provided with your LEUKOMAX® leukopak.
2. Spray down your BSC working space with detergent disinfectant, then 70% isopropanol. Clean the outside of your LEUKOMAX® leukopak with 70% isopropanol before moving into the BSC.
3. Cut the tubing on the LEUKOMAX® leukopak and drain the contents into a large, sterile container.
4. In 50 mL conical tubes, layer 30-35 mL of leukopak on top of 15 mL of density gradient medium.
    a. Alternatively, add 30-36 mL of leukopak to the tube first, then underlay 14 mL of density gradient medium using a 10 mL pipette tip.
5. Centrifuge conical tubes at 1200 x g for 15 minutes, 20°C, with the brake off. Wipe down the tubes with 70% isopropanol before returning them to the BSC.
6. Repeat steps 1-6 until all LEUKOMAX® leukopak contents have been centrifuged.
7. Aspirate the plasma layer to better access the white buffy coat layer, which contains the PBMCs.
8. Collect the buffy coat layer and transfer it to new 50 mL conical tubes. Due to the large number of cells, it is recommended to avoid pooling tubes until final resuspension.
9. Fill each tube to 50 mL with HBSS and gently invert to mix.
10. Centrifuge each tube at 800 x g for 10 minutes, 20°C, with the brake on.
11. Aspirate the supernatant, taking care to not disturb the pellet.
12. For a red blood cell lysis, follow the optional instructions below. Otherwise, continue to Step 13.
13. Fill the tube to 50 mL with HBSS and centrifuge again at 480 x g for 10 minutes, 20°C, with the brake on.
14. Aspirate the supernatant and resuspend in 10 mL HBSS.
15. Add HBSS up to 50 mL and centrifuge at 200 x g for 10 minutes, 20°C, with the brake on.
16. Aspirate the supernatant and resuspend in 10 mL HBSS, then up to 50 mL HBSS.
17. Perform a cell count and viability assessment.
18. Resuspend cells in the media of your choice and at your desired cell density. The PBMCs are now ready for use in your study.

Red Blood Cell Lysis (Optional)

1. After Step 11 in the above protocol, add 10 mL of ACK lysing buffer and resuspend the pellet by gently pipetting up and down. Incubate at room temperature for 5 minutes.
    a. If the cell pellet is larger than 3 mL, use up to 20 mL of ACK lysing buffer.
2. Resume the above protocol at Step 13.