Isolation of Peripheral Blood Mononuclear Cells (PBMCs) Using a Density Gradient Reagent

Preparation of Leukopak prior to performing isolation of PBMCs is crucial to ensure good starting material for your project.

As with other procedures, HemaCare scientists recommend that you closely follow the steps provided to ensure best results.

Materials Needed

- HemaCare Leukopak
- Density gradient reagent
- Washing buffer:
  - PBS without calcium or magnesium
  - 0.5% Human Serum Albumin (HSA)
  - 2mM EDTA
- 70% ethanol
- Sterile conical tubes - 50mL or larger
- Large sterile container to drain Leukopak

Equipment

- Biological Safety Cabinet (BSC)
- Serological and Micro-Pipettors
- Centrifuge

Notes

- Always wear personal protective equipment and use universal precaution when working with human-derived biological materials and liquid nitrogen.

Protocol

Preparation of Leukopak and density gradient separation

1. Measure and record initial product volume.
2. Spray down bag with 70% alcohol solution before placing in Biological Safety Cabinet (BSC).
3. Cut the tubing and drain Leukopak into a large sterile container.
4. Add 5-6mL of Leukopak material per 50mL conical. Label 50mL tubes.
5. Add PBS up to a total volume of 30mL per tube.
6. Using sterile 10mL serological pipettes, underlay 10mL of density gradient reagent under diluted Leukopak material in each tube.
7. Centrifuge tubes from step 6 for 30 min at 400 x g, 18-22°C, **brake set to OFF**.

Collection of Purified PBMCs

8. Upon completion of centrifugation, spray tubes down with 70% alcohol solution and transfer tubes back to the BSC.
9. Collect and pool the white colored PBMC layer on top of the density gradient layer into sterile, labeled, clean 50mL - 250mL tubes.
10. Centrifuge tubes containing collected PBMCs for 10 min at ≥450 x g, 18-22°C, **brake on**.
11. Aspirate supernatant.
12. Break up cell pellet by flicking or scrapping. Add working buffer to each cell pellet and mix to resuspend.
13. Pool cell suspension into labeled 50mL - 250mL conical tube.
14. Wash tubes with washing buffer. Collect in the labeled 50mL - 250mL conical tubes from step 13, using additional tubes as necessary.
15. Optional: wash tubes once more as in step 14.
16. PBMC sample is now ready for count and further study.

Tips and Tricks

1. The best concentration for effective antimicrobial treatment of surfaces is 70% ethanol. Sixty percent is too wet and takes too long to dry, rendering it less effective and 80% dries too fast to provide enough antimicrobial effectiveness.
2. When working in a BSC, you must use a lab coat with cuffs, and gloves. Outside the BSC, you should add goggles and a mask.
3. Ratio of 5mL Leukopak + 25 mL PBS + 10 mL density gradient is an optimized amount for HemaCare Leukopaks.
4. Centrifugation of 400 x g is optimal to obtain a clean buffy coat.
5. Centrifugation of 450 x g is optimal for removing the ficoll from the white cells.

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