



## PURIFICATION OF LYMPHOCYTES FROM PERIPHERAL BLOOD (PBL)

### Materials and Reagents

- 7-10 ml of blood collected in heparin lithium orin ACD (Acetate-Citrate-Dextrose)
- LYMPHOPREP tube 10 ml
- Conical tubes 15 ml
- RPMI 1640 Medium
- Complete RPMI 1640 medium: RPMI 1640 with a final concentration of : HEPES 25 mM, sodium pyruvate 2,5mM, glutamine 2mM, penicillin 100 IU/ml, streptomycin 100 µg/ml, 20% decompemented fetal calf serum
- Centrifuge

### Procedure

1. Dilute the blood 1:3 with RPMI.
2. Using a pipette, transfer the diluted blood to a tube, taking care to pour it on the walls.
3. Centrifuge 15 min at 2300 rpm.
4. Remove the supernatant and put it into a 15 ml conical tube.
5. Centrifuge 10 min at 2000 rpm.
6. Remove the supernatant.
7. Wash the pellet by resuspending it with a pipette in 5ml of RPMI 1640
8. Centrifuge 5 min at 2500 rpm.
9. Remove the supernatant.
10. Resuspend in 2ml of complete RPMI 1640.
11. Use 1 ml of the lymphocyte suspension for lymphoblastic transformation by EBV, and freeze the other half.