

PBMC Separation from Whole Blood

HANK'S and LSM are stored in the 4°C glass door fridge.

1. Allow HANK's and Lymphocyte Separation Media (LSM) to warm to room temperature before using these solutions.
2. Take out one FCS (Fetal Calf Serum) tube for each sample from the -20°C freezer and put on ice. Each tube contains 900µl of FCS. ***Perform the following steps in the fume hood.***
3. Invert Heparin blood tube gently to mix. Pour off blood into a 50ml conical tube.
4. Add 15ml of HANK solution to blood and mix well together.
5. Mix LSM by inverting the bottle several times. Underlay blood with 8ml of Lymphocyte separation solution, being careful not to disturb the layers.
6. Centrifuge at 2,100 RPM at room temperature for 20 minutes with the brake off.
7. Transfer the PBMC's (middle opaque layer) to a new 50 ml conical tube that contains about 2ml of HANK's solution (this prevents cell from clumping).
8. Add new HANK solution to the 50ml mark and mix well. Aliquot 10µl of cells into a 0.5ml eppendorf tube for cell counting with Trypan Blue. Use a dilution of 2:1 for cell counting, by adding 20µl of Trypan Blue to your 10µl of sample.
9. Centrifuge the tube at 1,200RPM for 10-12 minutes at room temperature with brake on. Pour off supernatant and tap tube to resuspend cells.
10. Add 100µl of DMSO to each FCS aliquot and add this mixture to your cells.
11. Aliquot your cells into a 1.8ml cryogenic vial.
12. Place into the cryogenic container and freeze in the -80°C freezer. You will move these to the -140°C later.