Tissue processing, freezing and thawing

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I. Blood

1. Spin down vials at 2000rpm for 15min at RT
2. Transfer ~6mL of plasma to a new 15mL conical tube and put on ice
3. Measure volume of blood and transfer to a conical tube
4. Using an equal amount of R10 media, wash the vials and transfer to the conical with the blood
5. In a Sepmate Conical, add 15mL ficoll
6. Slowly add the blood to the top of the ficoll, taking care not to mix the two
7. Spin down at 1200g for 10min at RT
8. Once separated, collect the upper layer of PBMCs
9. Place PBMCs into a new conical and bring the total volume up to 20mL with R10 media
10. Spin down vials at 1600rpm for 10min at RT
11. Pour off supernatant and mix in ~2mL ACK Lysis buffer
12. Let sit for 5min and then quench with 18mL of R10 media
13. Spin down vials at 1600rpm for 10min at RT
14. Resuspend in 10mL of R10 media
15. Count cells & freeze at 5-10 million/mL

II. Lymph Node Processing

1. Prepare media by adding 50µL DNase to 50mL R10 media
2. Add 10mL of prepared media to a culture dish
3. Place tissue in dish and gently cut away the fat
4. Rinse with 10mL of prepared media
5. FOR HPAP ONLY:
    a. Cut off a small piece of tissue (~3-5mm) and place in 50mL freshly prepared PFA/PBS solution
    b. Write date and time on the tube and store for ~24hrs at RT
    c. Transfer to 50mL conical with 80% EtOH and store at 4°C
6. Place tissue pieces in the cell strainer and smash with the top end of a 5mL syringe
7. Strain media in plate through the cell strainer into a 50mL conical
8. Rinse plate with remaining media and strain through the cell strainer into the conical
9. Spin down at 1600rpm for 10min
10. Wash cells with 10mL of R10 media
11. Count cells & freeze at 5-10 million/mL

III. Spleen Processing

1. Prepare media by adding 50µL DNase and 50µL Collagenase to 50mL R10 media
2. Cut tissue into pieces
3. Place tissue in a gentleMACS tube and dissociate
4. Incubate for 15min at 37°C
5. Repeat gentleMACS dissociation
6. Strain suspension through a 100µM filter
7. Spin down at 1600rpm for 10min
8. Pour off supernatant and mix in ~10mL ACK Lysis buffer
9. Let sit for 5min and then quench with 40mL of R10 media
10. Strain suspension through a 70µM filter
11. Spin down vials at 1600rpm for 10min at RT
12. Add 10mL ficol to a clean 50mL conical
13. Resuspend cells and very carefully add to the top of the ficol
14. Spin down at 2200rpm for 20min with the brake off
15. Resuspend cells with 10mL of R10 media
16. Count cells & freeze at 5-10 million/mL