BLACKBURN LAB PBMC PURIFICATION PROTOCOL FOR TELOMERASE ACTIVITY AND TELOMERE LENGTH MEASUREMENTS

1. PURPOSE

This protocol describes procedures to purify PBMCs using CPT tubes. The PBMCs pellets will be saved for telomerase activity and telomere length measurements.

At the end of the protocol, you should have 2 tubes of 1 million PBMC pellets in 1.5 ml screw cap tubes for telomerase activity measurements and 2 tubes of PBMCs with various cell numbers in 1.5 ml screw cap tubes for telomere length measurement. These will be stored at -80°C for batch analysis.

2. MATERIALS/REAGENTS/EQUIPMENT

Material	Vendor	Cat#
Vacutainer® CPT™ Tube with Sodium Citrate (8 ml Draw Capacity	BD	362761
DPBS	Invitrogen	14190
0.4% Trypan blue stain	Invitrogen	15250-061
10 ml sterile serological pipet	Fisher	13-678-11E
15 ml conical tube	BD	
Sterile 1.5 ml screw cap micro tube	Sarstedt	72.692.005
P1000 pipetteman	varies	varies
P200 pipetteman	varies	varies
P20 pipetteman	varies	varies
P1000 tips	Depending on pipetman	varies
P200 tips	Depending on pipetman	varies
Fine gel loading tip	Depending on pipetman	varies
Refrigerated microcentrifuge	Eppendorf 5430R or equivalent	
Tissue culture hood		
-80 freezer		
Vaccum trap	Set up on bench top	
Freezer boxes	82007-162	VWR

3. PROCEDURES

3.1. Collect blood into the CPT tube using the standard technique for BD Vacutainer® Brand Blood Collection Tubes. Invert tube 20-30 times to ensure proper mixing. The tube can be kept at room temperature on a platform mixer for up to 2 hours.

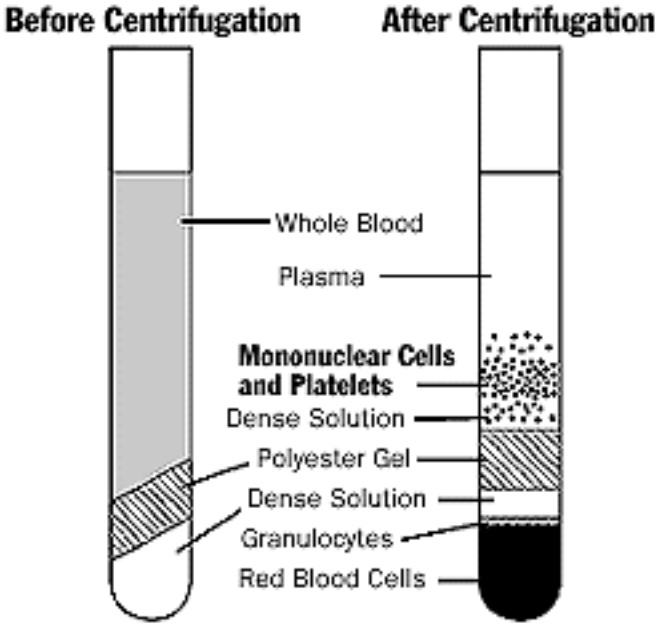
3.2. Remix the blood sample immediately prior to centrifugation by gently inverting the tube 8 to 10 times.

3.3. Centrifuge Tube/blood sample at room temperature (18-25°C) in a horizontal rotor (swing-out head) for 30 minutes at 1600 RCF (Relative Centrifugal Force) in a Sorvall Legend RT tabletop centrifuge (or equivalent).

3.4. After centrifugation, mononuclear cells and platelets will be in a whitish layer just under the plasma layer (**Figure 1**). Collect cell layer with a 1ml transfer pipette, carefully remove cells

from interface using a concentric circular motion and transfer to a 15 mL size conical centrifuge tube with cap. Collection of cells immediately following centrifugation will yield best results.

Figure 1: CPT tube before and after centrifugation



3.5. Add DPBS to bring volume to about 10 ml. Cap tube. Mix cells by inverting tube 8-10 times.

3.6. Centrifuge for 15 minutes at 300 RCF. Aspirate as much supernatant as possible without disturbing cell pellet.

3.7. Resuspend cell pellet by tapping tube with index finger, or gently pipetting up and down with a P1000 pipetman.

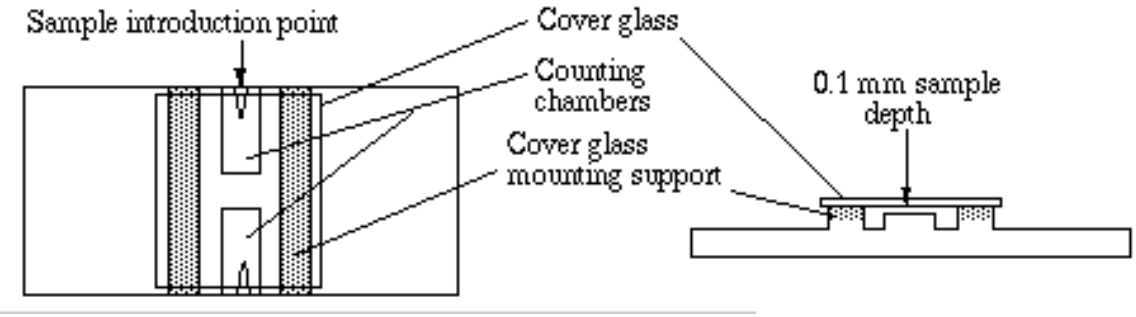
3.8. Add DPBS to bring volume to 10 ml. Cap Tube. Mix cells by inverting tube 8-10 times.

3.9. Centrifuge for 10 minutes at 300 RCF. Aspirate as much supernatant as possible without disturbing cell pellet. Resuspend cell pellet in 2 ml of DPBS. Gently pipetting up and down with a P1000 pipetman.

3.10. Dilute cell mixture to a 1:4 dilution by adding 30μL of cell mixture to 120 μL of 0.1% Trypan Blue.

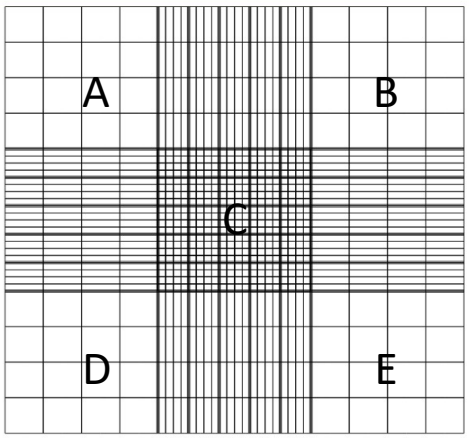
3.11. Place 10μL of dilution on hemocytometer (**Figure 2**).

Figure 2: Hemocytometer side view



NOTE: The hemocytometer consists of nine 1 mm squares divided into smaller squares. One 1 mm square represents a volume of 10^{-4} ml (**Figure 3**).

Figure 3: Hemocytometer top view



3.12. Count the number of cells (both live and dead) in a 1 mm square area. Count another 1mm square area if the number is below 100. Average the two counts to get your average cell number. To calculate your cell concentration per ml:

Cell concentration per ml = average cell number x 120/30 (dilution) x 10^4

NOTE: Count around 100-200 cells total (i.e. If one 1mm squares has too few cells, count more).

3.13. Record the number of live and dead cells and other information in the shared Google spreadsheet. Use the formula in the spreadsheet to calculate the volume required for 1 million cells for telomerase activity assay and the volume for telomere length assay.

3.14. To save PBMC pellets for TA, pipette the appropriate volume of cell suspension from the Google spreadsheet that contains 1 million cells in each of the two 1.5 ml screw cap tubes.

3.15. To save PBMC pellets for TL, split the remaining cell suspension into two 1.5 ml screw cap tubes based on the Google spreadsheet calculation .

3.16. Centrifuge for 5 minutes at 7000 rpm in the refrigerated microcentrifuge. Aspirate or discard as much supernatant as possible without disturbing cell pellet by using a thin pipet tip connected to a vacuum trap device. Store at -80 °C until batch assay.