

# Technical Notes—How to Count: Leuko Pak

Blood is obtained from normal healthy volunteers with collection of maximum three-blood volume on COBE Spectra™ apheresis machine.

## Reagent and Materials Required

- Sterile Room Temperature Washing Buffer: 500mL DPBS with 2mM EDTA
- Cold Washing Buffer: 500mL DPBS+10mL Newborn Calf Serum (NCS) or Newborn Bovine Serum (NBS)+2mM EDTA
- Cold Shipping Buffer: 500mL DPBS+8mL Bovine Serum Albumin (0.5%)+2mM EDTA
- Ammonium Chloride Solution (0.8% NH<sub>4</sub>Cl with 0.1 mM EDTA). Use approximately 10 to 15ml of ammonium chloride per 2-5x10<sup>8</sup> MNC per tube.
- 50mL Conical Tubes
- 5mL and 25mL Pipettes
- Latex gloves

## Equipment Required

- Class II Biological Safety Cabinet
- Beckman Centrifuge
- Pipettors
- Stainless Steel Surgical Scissors
- Stainless Steel Clamps

## Safety Precautions

- Protective gloves should be worn at all times.
- Use a safety pipetting device for all pipetting.
- NEVER PIPET BY MOUTH

## Procedure

1. Upon receipt of the Apheresis blood, verify that the product label is correct and donor information sheet is provided.
2. Remove the blood bag from biohazard bag. Place the apheresis bag in a Biological Safety Hood and examine the bag for leaks or clumps.
3. Mix the blood bag well and locate the blood transfer line.
4. Disinfect the blood transfer line with an alcohol swab and cut using sterile scissors.
5. Wipe the cut blood transfer line with an alcohol swab. Wait 1 minute until any remaining alcohol has evaporated. Carefully place 5ml of the blood in a sterile 50ml conical centrifuge tube and clamp the transfer pack to prevent any spills.

6. Take 25µl sample of blood and place into a pre-filled eppendorf tube containing 475µl Methylene Blue.
7. Add 80µl of DPBS into the mixing well (rounded non-sterile 96 well plate). Invert the eppendorf tube several times to mix the blood and Methylene Blue thoroughly.
8. Take a 20µl sample from the eppendorf tube and mix with 80µl DPBS. Take 20µl and load the Hemacytometer for the cell count.
9. Count the total number of mononuclear cells using *Methylene Blue Method*.

a. Methylene Blue Method Equation for Cell Count:  
# of cells/vial \_\_\_\_\_ /4 x 20 x d x \_\_\_\_\_ mL = \_\_\_\_\_ x 10<sup>6</sup>

b. To assess viability, use Trypan Blue Method. Mix equal volumes of cell to trypan blue (10µl:10µl) and dilute appropriately where necessary.

Equation for Viability:

# of cells excluded by trypan blue/ total number of cells x 100% = \_\_\_\_\_ %

## To Process PB-MNC from Leuko Pak

1. Count the cells from Leuko pak as detailed above.
2. Refer to "How to Deplete RBC," page 193.