

## Materials and Equipment

- Leukopak collected at HemaCare Donor Center
- HypoThermosol
- Washing buffer: PBS with 0.5% human serum albumin (HSA) and 2mM EDTA
- PBS without calcium or magnesium
- Sterile conical centrifuge tubes 250mL
- Sterile 1.8mL cryogenic vials
- Labels compatible for use in freezing and liquid nitrogen
- Transfer pack container (for processing &/or storage of blood or blood components, 300ml, 600ml or 1000 ml, Fenwal)
- 60 ml syringe
- Hemostats
- Biosafety cabinet (BSC)
- Pentra XL-80
- Flow Cytometer (Optional)
- Centrifuge
- Heat-sealer

## Method

- 1: Record the detail information of the leukopak and the total cell count by Pentra XL-80
- 2: Add Hypothermosol to ensure that the white blood cell (WBC) concentration is at the optimal concentration.
- 3: Calculate final volume for HypoThermosol Exchange
- 4: Print labels with the Catalog number and donor information on the Transfer pack container.
- 5: Spray the leukopak with 70% alcohol, wipe dry with paper towel and place into the BSC
- 6: Transfer the leukopak material into 250 ml conical tube(S).
- 7: (Optional) Mix the leukopak material and take 1 ml sample for phenotyping study (CD3, CD4, CD8, CD14,

CD16, CD19, CD56, CD45, PI).

8: Spin down the leukopak material at 600xg for 10 min at RT.

9: Spray bottles of HypoThermosol with 70% alcohol, wipe dry with paper towel and transfer HypoThermosol into BSC.

10: Transfer the conical tubes with leukopak material from centrifuge to BSC.

11: Discard the supernatant; loosen the cell pellet by gently tapping the tube.

12: Re-suspend the cell pellet with washing buffer.

13: Spin down the leukopak material in washing buffer at 600xg for 10 min at RT.

14: Discard the supernatant; loosen the cell pellet by gently tapping the tube.

15: Re-suspend the cell pellet with the amount of HypoThermosol calculated as Step 3.

16: Transfer the syringes and Transfer pack container into the BSC

17: Fill the transfer pack container with the leukopak cell suspension in HypoThermosol using the syringe.

18: Generate retention vials by transferring cell suspension in CryoStorCS10 into cryogenic vials (1ml to each cryogenic vial)

19: Triple seal the transfer pack container's tubing port with heat-sealer.

20: Send the HypoThermosol-Leukopak to shipping department for inspection and transportation

21: Transfer the cryogenic vial with cell suspension into CoolCells and transfer the CoolCells into -80°C freezer

25: Complete and sign production records and forms.

*To minimize the risk of injury or cross contamination, always wear personal protective equipment, including gloves, lab coat and goggles. For questions, please contact our scientists at (877) 944-4362.*

