



# STEM CELL LABORATORY (STCL)



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# **STCL-PROC-038**

## **CD45RA T CELL DEPLETION USING MILTENYI CliniMACS**

### **1 PURPOSE**

- 1.1 The CD45RA antigen is expressed on naïve CD4 and CD8 positive T cells, as well as on subsets of B cells, NK cells and monocytes. The overall frequency of CD45RA positive cells including CD45RA dim cells in PBPC is 55-75%. The CD45RA antibody recognizes the 220 kDa isoform of the leukocyte common antigen (LCA), a transmembrane tyrosine phosphatase.
- 1.2 This document describes the procedure for the preparation and automated separation/depletion of human CD45RA<sup>+</sup> T cells derived from G-CSF mobilized leukapheresis, utilizing the CliniMACS magnetic cell separation system

### **2 INTRODUCTION**

- 2.1 The CliniMACS system is a fully automated device designated for in vitro cell processing, selection, separation and/or depletion of specific cells. The CD45RA system is used for the depletion of human CD45 positive cells from heterogeneous cell populations. The CliniMACS system utilizes selective CD45RA monoclonal antibodies conjugated to super-paramagnetic particles. Super-paramagnetic particles are small in size (about 50nm in diameter) and are composed of iron oxide and dextran. The magnetic particles form a stable colloidal suspension and do not precipitate nor aggregate in magnetic fields. The CD45RA positive cells are specifically labeled during incubation with the CliniMACS CD45RA Reagent. After unbound reagent is washed from the suspension, the cells are ready for depletion in the automated continuous flow separation system. The CliniMACS system passes the antibody labeled cell suspension through a column in which strong magnetic gradients are generated. The non-targeted, magnetically labeled CD45RA positive cells are retained in the selection column while the unlabeled cells (target cells) flow through the column. Several automated washing steps are performed, collecting the CD45RA negative target cells in the cells collection bag.
- 2.2 When processing cellular products for transplantation, sterile technique should be used whenever feasible. Every precaution should be taken to minimize the possibility of contaminating cellular products.

### **3 SCOPE AND RESPONSIBILITIES**

- 3.1 The Medical Directors, Laboratory Supervisor, and Quality Manager are responsible for ensuring that the requirements of this procedure are successfully met.

### **4 DEFINITIONS/ACRONYMS**

- 4.1 mL                      milliliters
- 4.2 BSC                    biological safety cabinet
- 4.3 RPMs                  revolutions per minute

## **5 MATERIALS**

- 5.1 CliniMACS depletion tubing set, REF 261-01
- 5.2 CliniMACS CD45RA reagent, one 7.5mL vial
- 5.3 CliniMACS PBS/EDTA buffer, REF 700-25
- 5.4 600 mL transfer packs
- 5.5 Sampling site couplers
- 5.6 Plasma transfer sets
- 5.7 Luer/spike inter connector
- 5.8 Pre-system filter
- 5.9 25% Human serum albumin
- 5.10 Syringes, (1 mL, 5 mL, 10 mL, 20 mL, 30 mL, 60 mL)
- 5.11 Sterile Needles, various sizes
- 5.12 Sample tubes
- 5.13 SCD wafers

## **6 EQUIPMENT**

- 6.1 Biological safety cabinet (BSC)
- 6.2 CliniMACS cell separator
- 6.3 Hematology analyzer
- 6.4 Terumo SCD
- 6.5 Temperature controlled centrifuge
- 6.6 Plasma Expresser
- 6.7 Balance
- 6.8 Tubing heat sealer
- 6.9 Tubing stripper
- 6.10 Hemostats
- 6.11 Timer

## **7 SAFETY**

- 1. NA

## **8 PROCEDURE**

- 8.1 All work in this procedure should be performed in a BSC whenever possible using aseptic technique at all times.
- 8.2 Perform sample preparation and cell depletion at room temperature. Lower or higher temperatures will result in less purity and yield of the depleted cells.

- 8.3 Record lot numbers and expiration dates for all appropriate reagents/disposables on the worksheet *STCL-PROC-038 FRMI CD45+RA Depletion Worksheet* or on the appropriate lot sheet. Complete the worksheet as each procedure is performed.
- 8.4 Perform cell count, viability, HPCA and flow analysis (*to include percentage of CD45RA positive cells and the total number of CD45RA positive cells*) on the product per SOPs, recording results on the appropriate worksheets. Label this sample as **Sample A**.

**NOTE:** The capacity for the depletion of CD45RA positive cells using the CliniMACS CD45RA Reagent and the CliniMACS Depletion Tubing Set amounts to  $20 \times 10^9$  CD45RA positive cells out of a total cell number not exceeding  $50 \times 10^9$  (WBC) for processing.

- 8.5 Prepare working buffer reagent by adding 50 mL of 25% HSA to each of two liters of PBS/EDTA buffer. Mix well but gently to avoid foaming.
- 8.6 Manipulate the product to obtain the target volume of 200 mL.
- 8.6.1 If the product is greater than 200 mL, reduce the volume by centrifugation and plasma expression. Take care not to lose cells while expressing the plasma.
- 8.6.2 If the cell count is greater than  $200 \times 10^6/\text{mL}$  and the volume is less than 200 mL, dilute the product to reduce the cell count. Use autologous plasma and keep the maximum volume to 200 mL.

**NOTE:** If the leukapheresis product has to be stored overnight, it should be kept at controlled room temperature ( $+19^\circ\text{C}$  to  $+25^\circ\text{C}$ ). During storage, the concentration of leukocytes should never exceed  $200 \times 10^6$  cells per mL.

**NOTE:** An alternative starting cell source can be the negative cell fraction of a CliniMACS CD34 separation.

- 8.7 Label a 600 mL transfer pack “cell preparation” and place on the scale and tare the scale with the empty bag. Transfer the well mixed product to this bag and weigh it to obtain the volume.
- 8.8 Insert a plasma transfer set into a port of the buffer bag and sterile dock the PBS to the “**Cell Preparation**” bag and fill the product bag with buffer.
- 8.9 Heat seal the tubing between the bags, leaving enough tubing for several more sterile dockings and remove the PBS.
- 8.10 Mix the cells carefully and thoroughly
- 8.11 Centrifuge at room temperature at 200xg for 15 minutes with no brake.
- NOTE:** 200xg is equivalent to 800 RPMs in the Sorvall RC3C Plus and 1020 RPMs in the Allegra.
- 8.12 Sterile dock an empty 600 mL transfer pack labeled “**plasma waste**” to the “**cell preparation**” bag and hang on plasma expresser.

- 8.13 Remove supernatant completely without disruption of the cell pellet and disconnect the “*plasma waste*” bag. If necessary adjust the final volume to 95 mL, +/- 5 mL with PBS.
- 8.14 Mix the cells gently and thoroughly.
- 8.15 Inject one vial of cold CliniMACS CD45RA reagent. Withdraw the reagent into a syringe, adding a slug of air and inject into the sample, using the air to push the entire contents of reagent into the bag. Immediately set a timer for 30 minutes and incubate the cells at room temperature (+19°C to +25°C). Fill the bag with several more syringes of air and mix gently and thoroughly.
- NOTE:** The capacity for the depletion of CD45RA positive cells using the CliniMACS CD45RA Reagent and the CliniMACS Depletion Tubing Set amounts to  $20 \times 10^9$  CD45RA positive cells out of a total cell number not exceeding  $50 \times 10^9$  (WBC).
- 8.16 Rotate and invert the cell bag every 5 minutes during this antibody incubation to ensure that the reagent makes good contact with all cells.
- 8.17 At the end of the incubation period, sterile dock buffer to the cell preparation bag and fill the bag with buffer (*approximately 500 mL*). Heat-seal the tubing and remove the buffer bag.
- 8.18 Mix the cells gently and thoroughly.
- 8.19 Centrifuge at room temperature at 300xg for 15 minutes with no brake.
- NOTE:** 300xg is equivalent to 1000 RPMs in the Sorvall RC3C Plus and 1250 RPMs in the Allegra.
- 8.20 Label a 600 mL transfer pack “*wash waste*” and sterile dock it to the “*cell preparation*” bag.
- 8.21 Express as much supernatant as possible without disruption of the cell pellet and disconnect the “*plasma waste*” bag.
- 8.22 Re-suspend the cell pellet and mix gently and thoroughly.
- 8.23 Sterile dock buffer to the cell preparation bag and add buffer to produce a volume of 125 mL. Mix the sample gently and thoroughly.
- NOTE:** A maximum cell concentration of  $0.4 \times 10^9$  cells per mL of labeled/washed cells should not be exceeded for loading on the tubing set.
- 8.24 Remove samples for cell count, viability, HPCA and flow, recording results on the appropriate worksheets. Label this sample [Sample B](#).
- 8.25 Unpack the tubing set under the hood. Check luer lock connections on the columns. Luer locks must be tightly closed.
- NOTE:** CliniMACS tubing sets have been sterilized with ethylene oxide. Prior to opening the tray, inspect the package for any damage, punctures or tears which might indicate that the sterility of the set has been compromised.
- 8.26 Set up the CliniMACS

- 8.26.1 Switch on the instrument and press ENT to proceed to the program menu.
- NOTE:** Move the bar up and down by using the “0” and the “8” key. To proceed with the highlighted program, press “ENT”. Changes can be made to selections by pressing the “UNDO” key. To confirm choice and proceed, press “ENT”.
- 8.26.2 Choose **DEPLETION 3.1** by highlighting the name of the program and press ENT.
- 8.26.3 Select CliniMACS DTS and press ENT. Confirm the program and tubing set and press ENT to proceed.
- 8.26.4 Enter tubing set reference number (Ref. No. 261-01) and press ENT.
- NOTE:** The system is programmed to recognize reference numbers that do not correlate to the program chosen. The operator will be prompted to re-enter incorrect codes.
- 8.26.5 Input the sample parameters (*post cell labeling and washing*). Enter the WBC concentration of the sample and press ENT. Enter the percentage of labeled cells and press ENT. Enter the volume of the cell sample and press ENT. An internal calculation of labeled cells is performed, if correct, press ENT (*if not correct, press the “Undo” key to return to the previous screen*). Verify volume information and press ENT.
- NOTE:** From the total number of labeled cells internal calculations determine the number of separation stages, the amount of buffer needed for the depletion and the liquid volumes that will be collected in Non-Target Cell Bag, Buffer Waste Bag and Cell Collection Bag. If necessary, the cell collection bag of the CliniMACS depletion tubing set can be replaced with a transfer bag of appropriate size (*note weight of the new empty bag*) with the use of a luer/spike interconnector.
- 8.26.6 Attach the **Non-Target Cell Bag and the Reapplication Bag** to the right hand bag hanger.
- 8.26.7 Insert the Selection Column into the Selection Column Holder, making sure the “wings” are to the front. (*To avoid possible pinch injury, insert the column as follows: Hold the top and the bottom of the column between thumb and index finger, then carefully insert the column into the column holder.*) To proceed, press ENT.
- 8.26.8 The screen prompts to load valves 1, 2, 3, 4 and 5. The valves shown on the screen will be opened automatically.
- 8.26.9 Mount the tubing between valve No. 2 and the twist off cap into the liquid sensor. To assure proper operation, both the liquid sensor and the tubing being inserted must be dry. Carefully inspect both and dry with a lint free cloth if needed.
- NOTE:** Only insert tubing into open valves (*when the button is pushed inward*). If tubing needs adjustment after the valve has closed, do not pull the tubing without pressing the valve button to open the valve.

- 8.26.10 To proceed, press ENT.
- 8.26.11 Load the **pump tubing**.
  - 8.26.11.1 Open the pump door by lifting up at the left hand edge.
  - 8.26.11.2 Insert the upper retaining ring on the pump tubing into the retaining ring groove on the pump housing.
  - 8.26.11.3 Rotate the pump roller clockwise until the tubing is threaded between both sets of the tubing guide pins and the tubing fits snugly around the pump roller. Ensure the tubing is not pinched at the end of the guide pins.
  - 8.26.11.4 Insert the lower retaining ring on the pump tubing into the retaining ring groove on the pump housing.
  - 8.26.11.5 Repeat clockwise rotation of the pump roller to be certain that the pump roller moves freely.
  - 8.26.11.6 Close the pump door. **CAUTION:** During the cell selection sequence the pump will immediately stop the run whenever the pump housing is opened. If left open for more than 10 minutes, the instrument will abort the run in progress.
  - 8.26.11.7 To proceed, press ENT.
- 8.26.12 The screen prompts to load valves 6, 7, 8, 9 and 10. Load the tubing into the valves. Place the Buffer Waste Bag in the bag compartment and ensure the tubing is not compressed under the bag compartment lid. Press ENT to proceed.
- 8.26.13 Check all valves for proper tubing placement. Be certain that the tubing enters and leaves each valve through the enlargement at the inner end of the slot and is positioned in the center of the jaws of the valve. If tubing has to be readjusted, be sure to open the valve first. Check that none of the tubing is kinked or twisted. To proceed, press ENT.
- 8.26.14 In order to ensure the proper fitting of tubing in the valves, the CliniMACS will operate all the valves in sequence, twice. Watch and listen to ensure all valves are working properly.
- 8.26.15 Attach the **Selection Buffer Bag**.
  - 8.26.15.1 Remove the cap from the buffer spike on the tubing and connect it to the buffer bag using aseptic technique. Ensure that the septum is punctured, allowing free flow of liquid.
  - 8.26.15.2 Attach the buffer bag to the buffer bag hook on the bag hanger.
  - 8.26.15.3 Adjust the height of the buffer bag hanger. Raise or lower the hanger to accommodate the size of the buffer bag and to prevent severe bending of the tubing that could restrict flow and low enough to avoid the tubing connections being

stretched. It is important that the buffer bag is positioned higher than the reapplication bag and the non-target cell bag.

8.26.15.4 To proceed, press ENT.

8.26.16 Start the priming procedure by pressing RUN. The priming phase will take approximately 2.5 minutes and the priming status will be updated on the display. During this priming, check all tubing and connections for leaks or impediments to flow. If problems are found, press STOP. You will have 10 minutes to resolve the problem. Restart the process by pressing RUN.

**NOTE:** After 10 minutes the selection will be aborted. If you cannot resolve the problem, remove the tubing set and start over. Once priming has started, it is not possible to return to the instrument set-up procedure.

8.26.17 Perform a final check of all tubing and attachments to include:

8.26.17.1 Verification that there is fluid in all parts of the tubing set except for tubing above valves 2 and 3.

8.26.17.2 Verification that there is no excess air in the tubing set.

8.26.17.3 Verification that there is fluid in the reapplication, buffer waste and non-target cell bags.

8.26.17.4 Verification that there is no fluid in the cell collection bag.

8.26.18 To proceed, press ENT.

8.26.19 Perform the **integrity test for the upper part of the tubing set** by pressing 9. Clamp the tubing underneath the check valve of the non-target cell bag. Press RUN to begin the automated test sequence. Observe the tubing set for any leaks and replace if noted.

8.26.20 Perform the **integrity test for the lower part of the tubing set** by pressing 6. Press RUN to start the test sequence. Observe the tubing set for any leaks and replace if noted. Upon completion, remove the clamp underneath the check valve and press ENT.

8.26.21 Connect the **Cell Preparation Bag**.

8.26.21.1 Remove the twist-off cap from the tubing set.

8.26.21.2 Remove the cap from the spike of the pre-system filter and firmly insert the spike into the tubing set, ensuring the septum is punctured.

8.26.21.3 Remove the cap from the pre-system filter and the blunt end of the spike connector (*found with the tubing set*) and connect both parts.

8.26.21.4 Remove the other cap from the spike connector and spike the cell preparation bag. Gently squeeze the bag to ensure the spike has penetrated the bag.

- 8.26.21.5 Check the connection between the pre-system filter and the CliniMACS tubing set to confirm that the connection is secure.
- 8.26.21.6 Hang the cell preparation bag on the bag hanger.
- 8.26.21.7 **IMPORTANT:** Adjust the bag hangers so that the buffer bag hanger is in the highest position, the reapplication and non-target cell bags are in the middle position and the cell preparation bag is in the lowest position.
- 8.26.21.8 To proceed, press ENT.
- 8.26.22 Check the liquid sensor tubing to ensure that it has been properly inserted, that it is free of any external liquid and that it has not been dislodged during the loading procedure. To proceed, press ENT.
- 8.27 Press RUN to begin the automatic performance of the depletion procedure.  
**IMPORTANT:** At the beginning of the sequence, buffer is pumped upwards towards the cell preparation bag to fill the pre-system filter. Tap the side of the pre-system filter several times to remove any bubbles trapped in the filter.
- 8.28 When the run has finished, record the process code. Clamp or seal the tubing above the luer lock connecting the cell collection bag to the CliniMACS tubing set and transfer the bag to a BSC.
- 8.29 Weigh the cell collection bag to determine the final volume (*the weight of the empty cell collection bag attached to the CliniMACS depletion tubing set is 32 g*). Remove a well-mixed sample for cell count, viability, HPCA and flow. Label this sample [Sample C](#).
- 8.30 Heat seal the tubing above the luer lock of the remaining bags and remove.
- 8.31 To proceed, press ENT.
- 8.32 Remove the tubing set.
  - 8.32.1 Beginning with valves 6, 9 and 10 and working upwards, release the tubing from the valves by pressing the valves. Release the tubing from the liquid sensor and remove from pump.
  - 8.32.2 Release the column from the column holder.
  - 8.32.3 Dispose of the tubing set as biohazardous waste.
- 8.33 Shut down the CliniMACS.
- 8.34 Process the selected cells for transplant. If the reinfusion date is in the future, cryopreserve the cells as per standard operating procedures. If the cells are to be infused fresh, process them accordingly as per doctor's orders.

## 9 RELATED DOCUMENTS/FORMS

- 9.1 STCL-PROC-038 (FRM1) CD45RA+ Depletion Worksheet
- 9.2 STCL-PROC-038 (FRM2) Certificate of Analysis for CD45RA+ Depleted Donor Lymphocytes Infusion

- 9.3 STCL-PROC-038 JA1 Checklist for Depletion Tubing Set – DTS
- 9.4 STCL-PROC-038 JA2 Depletion Tubing Set Diagram
- 9.5 STCL-PROC-038 JA3 Separation Planner V2.40

## 10 REFERENCES

- 10.1 CliniMACS System User Manual, Miltenyi Biotec, Presidential Way, Woburn, Massachusetts, 01801.
- 10.2 Adult Bone Marrow Transplant Program Protocol Notebooks – internal protocols, Duke University Medical Center, Durham, NC.

## 11 REVISION HISTORY

Revision No.	Author	Description of Change(s)
02	B. Waters-Pick	<ul style="list-style-type: none"><li>• Added mL, BSC, and RPMS to Section 4</li><li>• Changed ml to mL throughout the document</li><li>• Updated punctuation and formatting throughout the document</li><li>• Added document numbers and additional documents to Section 9</li><li>• Changed STCL to Stem Cell Laboratory in the footer</li></ul>

## Signature Manifest

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