

Procedure Title: Hematopoietic Stem Cell Purging to Remove Neuroblastoma Tumor

Procedure #: HSC.B531.01

1. Abbreviations:

- A. STSX2 - used to transfer cells from a transfer pack to transfer pack
- B. STTA - used to pump cells from transfer packs through parallel magnets into empty transfer packs
- C. DIJS_T - used to multi-head peristaltic pump into transfer packs

2. Procedure:

A. Sample preparation

1. Remove the collection bag from the Stericell by Sebra sealing the entry tubing three times overlapping each seal and cutting the tubing at the middle seal.
2. After removal of the collection bag from the Stericell (see HSC.B522 for PBSC processing, HSC.B526 for bone marrow), remove an aliquot of cells in a laminar flow hood using a syringe and a 14-15 gauge needle for:
 - a. Test vials 90×10^6 cells* to a sterile 50 ml orange top tube
 - b. Cell counts 0.2 ml to a sterile snap cap tube
 - c. Immunocytology $30-40 \times 10^6$ cells** to a sterile 15 ml blue top tube

* only if there is no non purged backup frozen for infusion

** only if there is tumor present in the pre-harvest bone marrow

Weigh the bag on an electronic balance and record the weight on the worksheet. Calculate the total number of cells.

3. If the laboratory director or his designee has given instructions to save cells to be frozen as an untreated backup available for infusion, remove these cells before purging to the appropriate sized bag for centrifugation using an STSX2 (see procedure HSC.D211 for assembly instructions) or a sterile 60 ml syringe following the instructions below.

4. With the clamp closed, remove the cover from one luer end of the STSX2 and attach a 14-15 gauge needle.
 5. Remove the needle cover and insert the needle into the transfer pack containing the cells.
 6. Place the electronic balance in the hood and adjust the bubble level on the balance so that it is in the center of the red circle by adjusting the feet of the balance. Turn the balance on and wait for the display to clear.
 7. When the electronics are stable a small circle (stable circle) will appear in the top left of the display. Make sure that the weighing pan is clean and empty press the zero button to tare the balance.
 8. Place an empty transfer pack on the balance and insert a needle into the septum. of the sampling site coupler. When the stable circle shows, press the zero button to tare the bag.
 9. Open the clamp on the STSX2 and fill the bag to the appropriate volume and save for backup if necessary. Otherwise fill the bags for purging so that the final volume of cells and L15/HSA/GENT is no greater than 330 ml and the concentration of cells is $\sim 10 \times 10^6$ / ml. The concentration may be increased to a maximum of 15×10^6 / ml for bone marrow and 20×10^6 / ml for PBSC. The maximum volume per transfer pack including beads, cells, and media is 350 ml.
- B. Add goat anti-mouse / monoclonal antibody coated beads coated beads to bags with cells in L15/HSA/GENT for cycle one purging.
1. Check the Quality Control sheet for the immunobeads that are intended to be used. Ensure that the sterility and tumor removal testing results are acceptable prior to use. The criteria for acceptability follows:
 - a. The sample sent to CHLA Microbiology must have yielded a result of NO GROWTH for sterility testing for bacterial and fungal sterility.
 - b. The tumor removal test result must have been greater than 90% of tumor removed (Refer to HSC.D451) and tested within 3 months of the date of purging.

2. Based on the number of cells in each bag, calculate the appropriate number of monoclonal antibody coated beads to add to the bag. Use a ratio of one bead for each nucleated cell (i.e., 1:1 ratio).
 3. Mix the beads well by inversion, making sure all of the beads on the bottom of the tube have been placed in suspension. Attach a 14-15 gauge needle to a 30 ml syringe keeping the barrel of the syringe sterile.
 4. Remove the cap on the goat anti-mouse / monoclonal antibody coated beads tube, aspirate beads into the syringe, and inject the beads into each of the transfer packs. The barrel of the syringe **must be kept sterile** for all entries into the bead tube.
 5. After all of the beads have been added, cap the tube and invert the bags several times to mix the beads and cells. Place the bags into a container and rotate at approximately 20 rpm for 30 min. at room temperature.
- C. Place bags in a debulking (book) magnet and rotate for 5 minutes. If there is no further treatment with antibody coated beads, drain the cells into new 600 ml transfer packs following steps C.1. through C.3. and then proceed to step D.1.
1. Place the debulking magnet containing the beads and cells on its **side** in a laminar flow hood. Open an available port and with the clamp closed, spike into the bag with a solution transfer set
 2. Remove the cover from the luer end, attach a 14-15 gauge needle, and insert the needle into the septum of an empty transfer pack. Without laying the book magnets flat, remove the magnet from the hood, open the clamp, and drain the cells into the empty transfer pack.
 3. Remove a 0.2 ml aliquot from each newly filled bag into separate sterile snap cap tubes for cell counts. Weigh each transfer pack and record the weight on the work sheet.
 4. Estimate the number of beads to add for a second cycle of purging based on the average percent recovery of cells from the first cycle of purging.
 5. Repeat steps B.2. through C.2. before proceeding to step D.1.
- D. FINAL REMOVAL OF ANTIBODY COATED BEADS
1. After the cells have been drained into new 600 ml packs, place them back into the book magnets and rotate 7 minutes for the second

debulking. NOTE: prior to flow magnetic separation the cells must be debulked twice.

2. Based on a total volume of 600 ml of cells and media per transfer pack, prepare the appropriate number of transfer packs and DIJS_T's (see Procedure#: HSC.D211 for instructions) for final bead removal in a laminar flow hood as follows: clamp the tubing on an extension with "T", remove the cover, and insert the tip into the open port of a dual injection site. Clamp the exit tubing of a 600 ml transfer pack closed with an aluminum sealing ring and tape the exit tubing in a coil. Insert a sampling site cover into one of the ports.
3. In a laminar flow hood, with the clamp closed, spike an STTA- silicon tubing attached to a solution transfer set (see HSC.D211 for instructions) into the marrow bags still in the debulking magnets. Insert a 14-15 gauge needle onto the male needle adapter end of the STTA.
4. Hang the bags from a buret stand and pass the STTA through a column of 8 parallel magnets and then through a multi-head slow speed peristaltic pump.
5. Insert the needle from an STTA into the septum of a dual injection site that has been wiped with povidine followed by an alcohol wipe.
6. Pump cells at 5 ml/min into a 600 ml blood transfer pack until the transfer packs in the debulking magnets are empty. Change the transfer packs when the volume reaches 600 ml.
7. Remove the cell suspension bags from the parallel magnet device.
8. For instructions on cryopreservation of either bone marrow or peripheral blood stem cells see Procedure #: HSC.C101.