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Procedure: Product Cryopreservation

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I. PURPOSE

This procedure describes the proper method for autologous HPC, Apheresis product processing and the cryopreservation of cellular therapy products.

II. SCOPE

This procedure applies to Cellular Therapy Laboratory personnel performing cryopreservation of cellular therapy products. All CTL staff must comply with this policy.

III. STATEMENTS/REQUIREMENTS

Any exceptions to this policy must be approved by the CTL Medical Director.

IV. DEFINITIONS

AABB: Organization that advances the practice and standards of Transfusion Medicine and Cellular Therapies.

ACD-A: Anticoagulant Citrate Dextrose Solution, Solution A

BB: Blood Bank

BMT: Bone Marrow Transplant

CAP: College of American Pathologists

CTL: Cellular Therapy Laboratory

DIN: Donation Identification Number

DMSO: Dimethyl Sulfoxide

FACT: Foundation for the Accreditation of Cellular Therapy

HCT: Hematocrit

HPC: Hematopoietic Progenitor Cell

HSA: Human Serum Albumin

IUHPL: Indiana University Health Pathology Lab

MFV: Minimum Freeze Volume

MNC: Mononuclear Cells

MQC: Microbiology Control

MRN: Medical Record Number

NMDP: National Marrow Donor Program

PPE: Personal protective equipment

PVR: Post Volume Reduction

QC: Quality Control

SOP: Standard Operating Procedure

WBC: White Blood Cells

V. EQUIPMENT/RESOURCES

Equipment:

Laminar Flow Hood

Controlled Rate Freezer

Tube Sealer

Centrifuge Scale

Microscope

BB Refrigerator

Reagents/Solutions:

0.9% Sodium Chloride Injection USP, 100mL or 1000mL (normal saline)

25% Human Serum Albumin

Dimethyl Sulfoxide

Trypan Blue

Supplies:

Cryopreservation bags

Transfer Packs: 600mL, 300mL, 150mL

Luer lock syringes: 3mL, 10mL, 30mL, 50mL

Luer lock syringe cap

Couplers-plasma and DMSO resistant

Needles 16 Gauge

1.2 mL Cryovials

12 x 75mm Polystyrene Tubes

Pipette Tips 1-200uL

Pipette Tips 200-1000uL

Microscope Slides

22 x 22 mm Coverslips

Thioglycolate Tubes

Sterile Alcohol Wipes

Biohazard and sharps disposal containers

Sterile, single use scissors

Sterile, Single use forceps

Storage basin

Ice

Plastic zip-lock bags

Test tubes

VI. PROCEDURE

NOTE: Product Processing and Cryopreservation F-013 is referenced throughout this procedure. The performing technologist must initial all tasks and calculations completed during this procedure on F-013. If not explicitly stated in this SOP to initial, it is understood by technologist to initial after performing each step.

A. Product Receipt:

i. Refer to SOP PST 005 *Receipt of Cellular Therapy Products* for obtaining product, product receipt, product verification, and entry into CTL Activity Log. Also initial receipt on F-013.

B. Processing Preparation:

i. Put on proper PPE and prepare hood for processing. Refer to SOP QC 006 *Hood Quality Control and Disinfection.*

ii. Remove hanging tag and any extra tubing from the product bag and plasma bag, if applicable.

iii. Record the following on F-013:

1. Recipient and Donor Information from verified Request for Processing Order provided by BMT according to SOP PST 005 *Receipt of Cellular Therapy Products*.
2. Hood #, scale #, and heat sealer # being used.
3. Patient weight, collection date, end time, and time zone (from Apheresis product label)
4. Mobilization type from the AP/CTL Communication Sheet F-304
5. Sulfa allergy status from F-304.
    a. If the intended recipient has a sulfa allergy, highlight "Sulfa Allergy-Yes."
    b. The cryopreservative (DMSO) can elicit an allergic response due to its sulfoxide content.

iv. Weigh the product in its original bag and record the gross weight.

v. Calculate the estimated volume using the following formula.

(Product weight–bag and attachment weight)/1.027

Example: 433 mL – 45 mL/1.027 = 378 mL

1. See Attachment 1 for common bag and attachment weights.
2. The gross weight must be adjusted to exclude the weight of red blood cells. This is done by dividing the final weight by 1.027.

vi. Obtain and disinfect a storage basin. Place the product and plasma if applicable in the container and place under the disinfected hood.

Note: Only **ONE** product may be processed per hood. If concurrent plasma is received, the plasma bag must be stored with its corresponding product bag.

C. Product Processing:

i. All product processing must be performed using aseptic technique under a laminar flow hood according to SOP PM 018 *Aseptic Technique for Cell Processing.*

ii. Place the following under the hood:

1. Product and concurrent plasma if applicable.
2. Test tube labeled with interim labels (refer to PST 008 *Product and Reagent Labeling*) and "undil" (undiluted).

3. Thioglycollate broth tube along with label for a "pre" sample refer to RT 004 *Sterility Testing.*
4. 3 mL syringe
5. Coupler

iii. Mix and inspect concurrent plasma for acceptability if needed for freeze solution.
1. Plasma must be straw colored and not icteric, lipemic, or fibrous to be acceptable for use in the freeze solution.

iv. Mix the product and observe the product for clumping, clotting, discoloration, or anything else unusual.
1. If product appearance ok, proceed to step C.v.
2. If product appearance not ok, notify the CTL Medical Director and proceed as directed.
a. See Procedural Notes in section U.

v. Obtain a product sample:
1. With the coupler's clamp closed, if applicable, aseptically spike the product bag port with the coupler.
2. Using a 3 mL syringe, draw and flush the sample to mix before removing a sample with enough volume for MQC sample, CBC, ABO Rh typing, and a slide. Detach syringe, close the clamp on the coupler, and replace the cap.

vi. Charge the "pre" labeled Thio broth tube refer to RT 004 *Sterility Testing*.

vii. Transfer the remaining sample into the labeled test tube to be used for the following:
1. CBC and CBC dilution if necessary (refer to SOP RT 013 *Determining Product Parameters using Beckman Coulter Unicel® DxH 600 Analyzer*).
2. Flow cytometry (refer to SOP RT 012 *Flow Cytometric Acquisition and Analysis of CD34 and CD3*).
3. ABO/Rh determination (refer to SOP RT 009 *Autologous and Allogeneic Product Immunohematological Testing*).
4. Cell viability (refer to SOP RT 003 *Trypan Blue Cell Viability*) **Note:** applicable only for products that will be cryopreserved but not volume reduced on the same day of receipt).

viii. Use the sample remaining in the syringe to make a slide for the differential (refer to SOP RT 002 *Manual Differential*).
1. Stain the slide using the Hematek Slide Stainer (refer to SOP EO 005 *Hematek Slide Stainer*).
2. After the differential has been read (refer to SOP RT 002 *Manual Differential*), record results on F-013.
3. Calculate the MNC % using the following formula:
a. **MNC% = 100-(Band+Seg+Eo+Baso)**
b. **Example:** 100 - (7 + 15 + 0 + 0) = 78 % MNC

ix. Perform CBC.
1. Dilute the sample as appropriate and perform cell counts using the Coulter DxH 600 Analyzer (refer to SOP RT 013 *Determining Product Parameters using Beckman Coulter Unicel® DxH 600 Analyzer*).
2. Record the following on F-013:
a. WBC (use proper dilution to ensure result is w/in linearity).
b. HCT (use same dilution as reported WBC if possible).

x. Calculate the estimated total WBC count and record on F-013:

Total WBC = Estimated Volume (mL) x WBC ($x10^6$/mL)

Example : 378 mL x 154.00E+06/mL = 582.12E+08

xi. Perform ABO/Rh determination (refer to SOP RT 009 *Autologous and Allogeneic Product Immunohematological Testing*) and record on F-013.

1. ABO/Rh must match the previously reported type.
2. Any discrepancy must be resolved before continuing.

xii. Proceed to step D. if the product will be stored overnight or proceed to step E to begin cryopreservation.

D. Overnight storage:

i. Place a "Store at 2-6°C" label on the apheresis product and concurrent plasma if applicable (See Attachment 3).

1. Cross out "Store at room temperature 20-25°C" and initial and date.

ii. Place apheresis bag on top of the concurrent plasma bag (when applicable) in the storage basin. Store overnight at 2-6°C in a BB refrigerator.

1. Each patient product must be stored in a separate container on a separate shelf of the BB refrigerator.
2. Record storage location on F-013.
3. Record time placed in refrigerator.
4. Check "Yes" for overnight storage on F-013.

E. Cryopreservation Preparation:

i. Record processing tech initials, the hood #, scale #, and heat sealer # on F-013.

ii. Fill plastic ziplock bags with ice.

iii. Place bags in storage basin.

1. For products not stored overnight, place product collection bag and concurrent plasma bag (if applicable) on ice.
2. For products stored overnight:
   a. Verify the product and plasma bag (if applicable) have the correct patient name, MRN and DIN.
   b. Place cell bag and plasma bag (if applicable) on ice.
   c. Record time removed from refrigerator on F-013.
3. To prevent contamination, do not allow the ports from any bag to touch the ice filled bags.

iv. Place the iced product under the hood.

F. Evaluate product for concentration:

i. Estimate the Minimum Freeze Volume using the following calculation:

MFV = Total WBC x 10^8/5.00 x 10^8/mL

Example: 582.12E+08 / 5.00E+08/mL = 116 mL

ii. The MFV is the minimum total volume of cells and freeze solution needed to ensure that the cell concentration is a minimum of 0.50 x 10^8/mL and a maximum of 5.0 x 10^8/mL.

1. Due to low collection volume, some pediatric products may need to be cryopreserved at < 0.50 x 10^8 WBC/mL.
2. Approval from the CTL Medical Director and treating physician must be obtained prior to cryopreservation.

a. A deviation will be filed according to SOP ACQA 002 *Preparation and Control of Standard Operating Procedures, Policies and Forms*.

iii. As a general guideline, concentration of the product (refer to SOP PM 002 *Plasma Depletion (Concentration) of Cellular Therapy Products*) should be considered for the following:

1. If the estimated collection volume is 100 mL greater than the MFV

a. If the volume that can be removed is <100 mL contact the CTL medical director for approval prior to proceeding with plasma depletion.

i. Enter CTL Medical Director instructions of the F-013 comment section.
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2. When a donor requires multiple collections due to low $CD34^+$/kg, the total infusion volume may be significant. Product concentration should be considered to minimize the total infusion volume and the volume of DMSO infused.

3. If the intended recipient has a known sulfa allergy, the product should be concentrated to minimize the quantity of DMSO needed in the freeze solution, thus reducing the amount of residual DMSO given at infusion (refer to SOP PM 003 *Infusion of Cryopreserved Products*).

4. For allogeneic products, adverse effects due to plasma incompatibility may be minimized by concentration. Follow physician orders on Request for Processing Order or consult the CTL Medical Director (refer to SOP RT 009 *Autologous and Allogeneic Product Immunohematological Testing*).

iv. If concentration is not necessary check "No" for volume reduction on F-013 and proceed to step F.7.

v. Concentrate product per SOP PM 002 *Plasma Depletion (Concentration) of Cellular Therapy Products* if necessary.

1. Record the post volume reduction (PVR) WBC count/mL and check "Yes" for volume reduction on F-013.

vi. Weigh the bag and estimate the volume as in B.5.

Est. Volume = (Product weight–bag and attachment weight)/1.027

Example: [131 g – (30 g + 3 g)]/1.027 g/mL **=** 95 mL

vii. Calculate the new estimated Total WBC count as in C.10 using the PVR WBC and new estimated volume.

Total WBC = Estimated Volume (mL) x WBC ($x10^6$/mL)

Example: 95 mL x 612.00E+06/mL = 581.40E+08

viii. Calculate a new estimated MFV as in F.1 using the new volume and WBC.

MFV = Total WBC x 10^8/5.00 x 10^8

Example: 581.40E+08 / 5.00E+08/mL = 116 mL

Note: Calculations for steps F.6, 7, and 8 are estimates and should not be recorded on F-013.

G. Determine the number of freeze bags needed (see example below)

i. Determine estimated freeze volume using the new estimated volume from F.6: (round up to the nearest number divisible by 10)

Estimated volume/0.8 = Est. Freeze volume

Example: 95 mL / 0.8 = 119 mL

ii. Compare the estimated freeze volume with estimated MFV from step F.1 or F.8 if the product was concentrated.

iii. Choose the larger of the two values (estimated freeze or MFV).

iv. Divide the value by the maximum bag volume and round up to the next whole number. This is **minimum** number of freeze bags required.

v. Check that the following conditions are met.

1. Estimated MFV is evenly divisible into the number of bags. If not, increase the estimated freeze volume until evenly divisible by 10. **Note: be sure the estimated freeze volume does not exceed the maximum allowable volume/bag.**
2. Protocol for the patient's treatment plan (Attachment 2) i.e. "freeze in even number of bags" has been followed.
3. Total freeze volume must be within validated freeze bag range. See Attachment 1 for limits.
4. Specific instructions given by the CTL Medical Director and/or the treating physician. Refer to F-013 and Request for Processing regarding the number of freeze bags has been followed.

MFV (round up to nearest number evenly divisible by 10)	= 116 → 120

Example:

Estimated volume of collection	= 95 mL
95 ÷ 0.8 (round up to nearest number evenly divisible by 10)	= 119 → 120 mL
Note: Use the larger of the two values (MFV and volume/0.8)	
To determine how many freeze bags are needed:	
Estimated freeze total volume	= 120 mL
÷ 70mL or (20mL for peds) maximum bag volume (round up to nearest whole #)	= 120/70 = 1.7 →2
Minimum # of bags needed to freeze collection volume	**=** 2
Check to see if estimated freeze volume is evenly divisible by minimum bag#. If not, Increase estimated freeze volume by increments of 10 until it is evenly divisible. ****be sure not to exceed maximum bag volume of 70mL****	120/2 = 60 **Can freeze in 2 bags**
Refer to protocol for this diagnosis (Attachment 2; also see "Special Instructions" section on F-013)	→ (Must be frozen in an even# of bags)

H. Distribute product equally into freeze bags:

i. Label the appropriate number of freeze bags with an interim label with each bag numbered sequentially.

ii. **If using Miltenyi brand bags:** Heat seal to remove the white capped cell (out) port and one of the blue capped (in) ports, leaving the DMSO safe tubing and one blue capped cell port with a roller clamp attached to the freeze bag.

iii. Using a 50mL syringe, remove the maximum volume of one syringe that may be added to all freeze bags. Add this volume to the first freeze bag and place the bag on ice. Repeat for each additional freeze bag.

iv. Determine the next syringe volume and add to all freeze bags sequentially.

v. Repeat until the total collection volume has been equally distributed to all freeze bags and return all of the bags to ice.

vi. If desired, some of the excess air may be removed from the freeze bags using a syringe during the distribution process.

vii. For products that have been stored overnight and for products that have not been volume reduced, reserve 1-2 drops in the final syringe for a viability check according to SOP RT 003 *Trypan Blue Cell Viability*.

viii. Tally the actual measured volume distributed and record on F-013.

ix. **If using Miltenyi brand bags:** Heat seal the remaining blue capped cell ports once all cells have been transferred to freeze bags, leaving only the DMSO safe tubing and port on the bags.

Example:

Volume in first syringe	30 mL
+ Volume in second syringe	+ 22 mL
= Total volume in each freeze bag	= 52 mL/bag
x Total number of freeze bags	52 x 2 bags
Actual Measured Volume	= 104 mL

x. Store all freeze bags, plasma (concurrent or PVR) and the freeze solution bag on ice so that they will be properly cooled when ready to prepare the freeze solution.

1. The collection bag should be used for preparing freeze solution unless the product was volume reduced.
2. If the product was volume reduced, use the transfer pack in which the cells were centrifuged for the freeze solution.

I. Cell Viability: (refer to SOP RT 003 *Trypan Blue Cell Viability*)

i. Viability may be performed at any time during the procedure,, but must be performed prior to freeze solution addition. Record results on F-013.

1. For products that have been stored overnight but not volume reduced, use the sample from H.7
2. For products stored overnight AND volume reduced, use the diluted PVR CBC sample from SOP PM 002 *Plasma Depletion (Concentration) of Cellular Therapy Products*.
3. For products being cryopreserved on the same day of receipt, the CBC sample from C.7 can be used for the viability sample.

J. Determine freeze solution content:

i. Calculate Total WBC (final) using the following calculation and record of F-013:

Actual Measured Volume x WBC count/mL = Total WBC

Example: 104 mL x 612.00E+06/mL = 636.48E+08

1. For products that have not been concentrated, use the original WBCcount/mL.
2. For products that have been concentrated, use the PVR WBC count/mL.

ii. Calculate recovery % if applicable and record on F-013.

Processed Product Total WBC / Collected Product Total WBC x 100%

Example: 636.48E+08 / 581.40E+08 x 100 = >100%

1. Recovery % must be ≥ 80% (refer to SOP PM 002 *Plasma Depletion (Concentration) of Cellular Therapy Products*)
2. Record the ID# of the centrifuge used.

iii. Calculate actual MFV.

1. See F.1 to calculate; replace estimated Total WBC with final Total WBC from J.1 and record value on F-013.

iv. Determine actual total freeze volume.

1. Divide the actual measured volume by 0.8.
2. See G.1 to calculate; replace estimated volume with Measured Volume.
3. Compare with the actual MFV from and choose the larger of the two values.
4. Round up the number until it is evenly divisible by 10 and also evenly divisible by the number of freeze bags. **This is the total freeze volume.**

v. Determine freeze solution volumes. (see example below)

1. Subtract 10% from the total freeze volume.
2. Subtract the actual measured volume from the subtotal.
3. The remaining value is the amount of plasma/HSA to be added.
4. Verify that the total freeze solution to be added is equally divisible by the number of freeze bags and is a whole number.
    a. If not equally divisible, increase the total freeze volume by 10 mL and re-calculate the freeze media calculations.
    b. Repeat until the total freeze solution added per bag is a whole number equally divisible by the total number of freeze bags.

vi. Verify plasma/HSA to DMSO ratio is ≥1:1.

1. If it is not, add 10 mL to total freeze volume and repeat calculations.
2. Repeat until the volume needed to satisfy the ≥1:1 ratio is reached.

Example:

Actual freeze total volume	= 130 mL
- 10% DMSO	13 mL
(Subtotal)	= 117 mL
- Measured volume of collection	- 104 mL
Estimated volume of plasma (or 5% HSA solution or both) in the freeze solution	**= 13 mL (≥ 1:1 ratio)**

vii. When the above criteria have been met, record all freeze media calculations on F-013. Also record the volume/bag calculations.

viii. Document DMSO volume, lot #, and expiration date on F-013.

K. Determine the source of plasma and/or 5% HSA:

i. The plasma/HSA volume needed for the freeze solution may come from one of the following:

1. All donor plasma.
   a. Choose PVR plasma first if available. It is preferable since it may contain a minimal number of WBCs.
   b. Choose concurrent plasma second if available and it was determined to be acceptable.
2. Donor plasma (concurrent or PVR) and the balance needed from 5% HSA solution.
3. Donor plasma and the balance needed of 0.9% NaCl (normal saline) provided that the total volume of donor plasma is >8% of the total freeze volume.
4. 5% HSA only

ii. Document the volume(s) used of plasma, 5% HSA, or both on F-013.

iii. If 25% HSA and/or saline were used, document the volume, manufacturer, lot #, and expiration date.

iv. Circle "auto", "allo", or "N/A" plasma on F-013 if applicable.

L. Determine WBC concentration and record on F-013:

WBC Concentration = Total WBC x10^8/ total volume mL

Example: 636.48E+08 / 130 mL = 4.90E+08/mL

i. This value must be within defined limits of 0.50 x 10^8/mL to 5.0 x 10^8/mL.

ii. If all freeze criteria have been met and all calculations have been performed properly, the calculated value will be within this range. If it is not, a calculation error may have occurred; recalculate all values. If unable to resolve, consult a 2nd technologist to determine where an error might have occurred.

M. Calculate MNC and MNC/kg.

Use the following calculations.

Total MNC = Total WBC x %MNC

MNC/kg = Total MNC/patient wt.

Example:

Total MNC = 636.48E+08 x 78% = 496.45E+08
MNC/kg = 496.45E+08 / 103.3 kg = 4.8E+08

i. Record and initial F-013.

N. Calculate final flow cytometry results:

i. Use the (final) Total WBC (refer to SOP RT 012 *Flow Cytometric Acquisition and Analysis of CD34 and CD3*).

ii. Use patient weight from collection label.

O. Verify calculations:

i. Consult a 2nd CTL technologist to verify the accuracy and completion of all freeze media calculations and initial F-013 after review has been completed.

ii. The 2nd tech also ensures that product labels including circular of information and biohazard labels have been completed and checked for accuracy and that viability testing has been completed.

NOTE: FREEZE SOLUTION CALCULATIONS MUST BE REVIEWED PRIOR TO ADDITION TO CELLS.

P. Prepare freeze solution:

i. Determine actual plasma and/or 5% HSA solution volume to add to the freeze solution.

1. Add 1 mL extra for each freeze bag to the calculated plasma/HSA volume. This added volume will be lost to tubing during the addition of the freeze solution and ensures that the correct volume of freeze solution per bag will be available. **Note:** This value is not recorded on F-013.
2. On page 10 of F-013, record the actual plasma volume, total DMSO volume, and volume of freeze solution/bag. Place the note where it will be visible while working under the hood (but not under the hood). This eliminates the need to come out of the hood to check calculated volumes and re-enter the hood, thus reducing the possibility of contamination.

ii. Prepare 5% HSA if necessary:

1. Obtain a bottle of 25% HSA, a 100 mL or 1000 mL bag of normal saline, and an appropriately
2. sized transfer bag according to the table below.

Volume Needed	Transfer Bag Size	25% HSA	0.9% NaCl (saline)
< 50 mL	150 mL	10 mL	40 mL
51 - 100 mL	150 mL	20 mL	80 mL
101 - 200 mL	300 mL	40 mL	160 mL

3. Record total volume used in mL, manufacturer, lot number, and expiration date for the 25% HSA and the saline on F-013.
4. Label the transfer bag as 5% HSA. Spike both the transfer bag and the saline bag using plasma transfer sets with female luer adapters.
5. To add saline: Use a 50mL syringe to remove the desired saline volume and add to the transfer bag.
    a. Label saline bag with open date and time and tech initials. Saline expires 12 hours after opening.
6. To add 25% HSA:

a. Obtain the appropriate size syringe for the volume of 25% HSA needed and attach a 16 gauge needle. Wipe the stopper of the 25% HSA bottle with an alcohol pad. Carefully puncture the stopper and withdraw the needed volume. Remove the needle using forceps and discard into the sharps container.

b. Add the measured volume of 25% HSA to the transfer bag containing the saline and thoroughly mix.

7. The 25% HSA solution expires four hours after opening of HSA bottle; the prepared 5% HSA solution expires four hours after preparation.

8. Place 5% HSA solution on ice. Cool for a minimum of 10 minutes before proceeding.

iii. Obtain the pre-cooled bag that will be used for the freeze solution and label the bag "frz soln". Use a DMSO resistant coupler.

iv. Obtain the appropriate size syringe and remove the volume of plasma and/or 5% HSA solution or saline needed. Remember to include the 1 mL/freeze bag extra freeze solution. Add to the bag labeled "frz soln". Place in container with ice.

v. Obtain the appropriate size syringe for the volume of DMSO needed and attach a 16 gauge needle. Wipe the stopper of the DMSO bottle with an alcohol pad. Carefully puncture the stopper and withdraw the needed volume. Remove the needle using forceps and discard into the sharps container.

vi. Attach the syringe containing DMSO to the coupler of the freeze solution bag.

1. While gently mixing the chilled plasma and/or 5% HSA solution, slowly add DMSO while ensuring that the solution temperature does not increase.

2. The addition of the DMSO causes an exothermic reaction. Do not add the DMSO too quickly and do not use plasma and/or 5% HSA solution that has not been properly pre-cooled.

3. If the solution begins to feel warm, place the bag on ice to cool. After the freeze solution has cooled, continue adding DMSO. Repeat cooling if necessary until all DMSO is added.

vii. Place freeze solution bag on ice. Set a timer for 15 minutes to allow for proper pre-cooling of the freeze solution.

Q. Prepare for freezing process:

i. Select appropriate freeze canister(s) for the product (refer to SOP PST 004 *Product Storage, Inventory Tracking and Disposal*). One canister is needed per freeze bag. Record the canister ID for each bag to be frozen and the freezer number on F-013. Each pediatric bag needs a pediatric canister along with a regular canister.

ii. Prepare the product freeze labels (refer to SOP PST 008 *Product and Reagent Labeling*).

1. Labels must be checked for accuracy against the verified Request for Processing Order from BMT by a 2nd technologist; initial F-013.

iii. Prepare labels for the freeze curve, cryovial inventory log, and freezer inventory log.

1. On each freezer inventory log label, write the bag number and canister location.

2. Place the labels on the CTL whiteboard (refer to SOP PST 004 *Product Storage, Inventory Tracking and Disposal* and SOP PST 007 *Infectious Disease Marker Testing*).

3. Initial F-013.

iv. Prepare the freeze chamber and place a cryovial rack, appropriate canisters and cassette racks (as needed), and any dummy bags in the chamber (refer to SOP EO 001 *Operation of Cryomed Controlled Rate Freezers*).

v. Obtain the assigned Thio tube, fungal cryovial, labeled cryovials, and a 50mL syringe. Set under the hood for use later.

R. Add freezing solution to bags:

i. After the freeze solution has chilled for a minimum of 15 minutes, it may be added to the cells. Cooling the solution prevents DMSO toxicity to the cells.

ii. Start selected freeze chamber (refer to SOP EO 001 *Operation of Cryomed Controlled Rate Freezers*).

iii. Attach a 50mL syringe to the coupler sampling site on the freeze solution bag and remove the calculated freeze solution/bag volume. Detach syringe and place the freeze solution back in the ice container.

iv. **If using Miltenyi brand bags:** Ensure the DMSO safe tubing is clamped with forceps before removal of the cap.

v. Attach the syringe with freeze solution to luer adapter of the first freeze bag.

    1. Note the time of the start of the addition of the freeze solution and record on the F-013.

vi. Gently rock the freeze bag continuously while slowly adding the freeze solution, ensuring that the temperature of the cells does not increase.

    1. If the temperature of the cells increases, place the freeze bag on ice until cooled; remove and continue to add the freeze solution.
    2. Repeat as necessary until all freeze solution has been added. Reduce the rate of freeze solution addition if repeated cooling is needed.

vii. Remove excess air from the freeze bag:

    1. With the syringe still connected, invert bag and tap ports to release air bubbles. Maneuver the bag so that excess air bubbles gather near the port then draw the excess air out of freeze bag into syringe.
    2. Sometimes more air is in the freeze bag than can be expelled with one syringe; if necessary, forcep the tubing, detach the syringe from the freeze bag and expel the air with an alcohol pad covering the top of the syringe. Re-connect, remove the forceps, and continue to remove all excess air.

viii. Remove sample for MQC and cryovial storage:

    1. If only 1 bag is being frozen:
        a. Remove approximately 1.5 mL so that each of 2 cryovials may be filled with 0.5 mL of product.
        b. With the remaining sample, charge the "post" thio tube and fungal cryovial each w/3-5 drops.
    2. If multiple bags are being frozen:
        a. From one bag, remove approximately 0.5 mL of well-mixed cell suspension using the attached syringe and transfer to a labeled cryovial.
        b. From a different bag, remove approximately 1.0 mL
        c. Add 0.5 mL to the second cryovial
        d. With the remaining sample, charge the "post" thio tube and fungal cryovial each with 3-5 drops.

ix. Place cryovials for storage on ice or in the pre-cooled freeze chamber while processing is completed.

x. Heat seal the tubing on the freeze bag in a direction that is parallel to the freeze bag and as close to the port opening as possible.

    1. Make another seal above the first one, then, using the single use, sterile scissors, cut in between the seals as close to the first seal as possible.
    2. Inspect the heat seal and re-seal if necessary to ensure a good weld.

xi. Place the bag back into the ice and repeat this process with the remaining bags.

xii. Thoroughly remove all moisture from the freeze bag. Apply final product label and bag tag (refer to SOP PST 008 *Product and Reagent Labeling*).

S. Refer to SOP EO 001 *Operation of Cryomed Controlled Rate Freezers* for the following:

i. To start the freeze program. (Each freeze program run is approximately 50 minutes.)

ii. To remove products from the freezer and store the product.

iii. Freeze curve documentation.

iv. Document and initial each step on F-013.

T. Clean work area:

i. Discard waste appropriately according to Indiana University Health Waste Segregation and Disposal Procedure and document on F-013.

ii. Clean hood (refer to SOP QC 006 *Hood Quality Control*).

iii. Re-stock supplies if needed.

iv. Place the charged MQC thio broth and fungal cryovial in a biohazard bag and send to the IUHPL Microbiology Lab via the pneumatic tube system (refer to SOP RT 004 *Sterility Testing*).

1. MQC samples may be sent to the Microbiology Lab at the end of the day if other MQC samples will be collected later.

v. Once the freeze and freeze curve has been filed, perform a final paperwork check verifying that all initials and calculations have been completed.

vi. Place the file in the designated area for 2nd tech review.

U. Procedural Notes

i. For products that do not pass visual inspection proceed as follows after consulting the medical director.

1. If clumping or clotting is observed and does not return to suspension, ACD-A may be added with the CTL Medical Director's authorization.

a. The volume of ACD-A added may not exceed 10% of the total product volume.

b. Obtain ACD-A from IU Health Apheresis.

c. Record volume, manufacturer, lot #, and expiration on form F-013.

d. Also note that additional ACD-A was necessary in the section for "Special Instructions/ Comments/Samples Removed for."

ii. If discoloration is observed, bacterial or fungal contamination may be suspected. At the CTL Medical Director's direction:

1. Order a Stat gram stain; report findings to CTL Medical Director and proceed as directed.

2. Document the date and time of notification and action taken, on F-013 in the section for "Special Instructions/Comments/Samples Removed for."

iii. Complete a Nonconformity of Products and Materials / Dose Limitations (F-081) for any of the above situations (refer to SOP QC 022 *Nonconformity of Products and Materials / Dose Limitations* and record on F-013.

VII. CLINICAL SIGNIFICANCE/SPECIAL CONSIDERATIONS

None

VIII. REFERENCES

AABB Technical Manual, current edition.

AABB Standards for Cellular Therapy Product Services, current edition.

CAP Standards, current edition.

FACT Standards for Cellular Therapy Product Collection, Processing, and Administration, current edition.

IX. FORMS/APPENDICES

F-013 Product Processing and Cryopreservation

F-304 Apheresis/Cellular Therapy Lab Communication Sheet

F-081 Nonconformity of Products and Materials/ Dose Limitations

Attachment 1: Freeze supply weights and limits
Attachment 2: Freezing Plan According to Patient Protocol
Attachment 3: Store at 2-6°C

X. APPROVAL BODY

None

Procedure #:

PM 001