Purified Human Pancreatic Islets, Supplementary Purification, OptiPrep Procedure and Record – A Standard Operating Procedure of the NIH Clinical Islet Transplantation Consortium

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Document Title:

PURIFIED HUMAN PANCREATIC ISLETS SUPPLEMENTARY PURIFICATION, OPTIPREP PROCEDURE & RECORD

1.0 MATERIALS

See Production Batch Record Section 3.1.3, Raw Materials and Purchased Reagents, and SOP 3106, B10, Purification Density Gradients preparation record.

2.0 PROCEDURE

2.1 Bring the Supplementary Purification Islets from PBR Section 8.5 to approximately 250 mL with CIT Purification Solution and gently re-suspend them. Seal the tube and place it at 2ºC to 8ºC for 30 to 50 minutes while preparation for Supplementary Purification occurs. Then proceed to the Supplementary Purification.

2.2 COBE 2991 Preparation

Set up the COBE according to the Operational Manual and the institution's procedures. The COBE must be refrigerated or placed in a cold room.

- Prepare High (1.10 g/mL) and Low (1.06 g/mL) CIT Purification Density Gradients according to SOP 3106, B10, and file the records of their preparation with this Production Batch Record.
- Label 13 X 250 mL conical tubes with the COBE run number and "W1" and fraction numbers 1 through 12 (See tables in Section 8.3). Label a 14th 250 mL conical tube with the COBE run number and "Bag."
- Fill tubes 1 through 12 with 225 mL of CMRL 1066, Supplemented, and store at 2ºC to 8°C.

Verified by: _____ **Date:** _____

2.3 COBE 2991 Procedure – Gradient and Tissue Loading

2.3.1 Assemble the COBE bag onto COBE cell processor according to institution's procedure. Place clamps near the main line on all colored tubing except one line to be used for loading the COBE bag.

2.3.2 Place gradient-maker on magnetic stir plate and aseptically connect one end of size 16 tubing to gradient-maker and the other end to green tubing of the COBE bag.

2.3.3 Place a sterile stir bar into the left chamber (next to outlet) and turn on the stir plate.

2.3.4 Run tubing through pump and set pump to 60 mL/min.

2.3.5 Sanitize the exterior of all solution bottles before placing in the hood.

2.3.6 Pour 120 mL of the High Density Gradient into the left chamber of the gradient maker.

Islets Lot Number: _____

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2.3.7 Pump the bottom layer into the COBE Bag then stop the pump.

2.3.8 Remove excess air from the COBE bag by pressing Superout while unclamping the red tubing. Press the Hold button once the Bottom Gradient has reached the T (junction of red/green tube). Re-clamp the red tubing line and press the Stop/Reset button.

2.3.9 Begin loading the continuous density gradient into COBE bag.

- Pour 125 mL High Density Gradient (1.10 g/mL) in the left chamber (nearest the outlet) of the gradient maker. Open and close the port between the two chambers just enough to fill the opening.
- Pour 125 mL Low Density Gradient (1.06 g/mL) in the right chamber of gradient maker (away from outlet)
- Open the port between the chambers, set pump to 20 mL/min and load gradient up to the T of the COBE bag tubing. Stop the pump when the gradient has reached the T-connection.

Note: Observe the gradient maker to ensure that gradients are mixing during the continuous gradient loading.

2.3.10 Start the COBE and ensure the centrifuge speed is 1800 to 2000 rpm.

Centrifuge Speed: _____ rpm

Recorded by: _____ **Date:** _____

2.3.11 Load the continuous gradient by unclamping the green tubing and starting the pump. Load the entire 250 mL of continuous gradient at 20 mL/minute.

2.3.12 When all of the gradient has been loaded, stop the pump just as the last portion of the gradient enters the tubing attached to the gradient maker.

Note: COBE must remain spinning during the rest of the purification process. If abnormal signs appear from rotating seal (e.g. leak, unusual noise, burnt smell, etc.), replace COBE bag and make new density gradients.

2.3.13 Aseptically remove the tubing from gradient maker port and move to the beaker with tissue. Reverse the pump to purge the air.

2.3.14 Load the Supplementary Purification Islets (Section 8.5.2) with the pump at a setting of 20 mL/min. Gently swirl the beaker to keep the tissue well suspended during the loading.

2.3.15 To ensure tissue does not back-up on the gradient (a heavy tissue line observed on the gradient), periodically turn the pump off allowing tissue to enter the gradient and then turn the pump back on again. Repeat as necessary every 1 to 2 minutes.

2.3.16 As soon as the tissue is loaded, add 30 mL of additional CIT Purification Solution to the 250 mL beaker to rinse. Load this rinse onto the COBE.

2.3.17 After the last portion of the rinse has entered the COBE bag, stop the pump.

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2.3.18 Vent the system by carefully unclamping the red tubing. Re-clamp the tubing when liquid (capping solution) is approximately one inch above the ceramic seal.

Note: Air left in the ceramic rotating seal can cause seal failure which may lead to leaking, seal occlusion and possible system shutdown due to overpressure during Superout.

2.3.19 Clamp the green line and allow the COBE to spin for 3 minutes. Record data on the Data Log for the Re-purification COBE run, below.

Verified by: _____ **Date:** _____

2.4 COBE 2991 Procedure – Tissue Collection

2.4.1 During the 3 minute spin disconnect tubing from the pump. Prepare for collection of tissue fractions.

2.4.2 Verify that the Superout Rate is set at 100 mL/min.

2.4.3 After 3 minute spin, slowly remove the blue clamp on the green line and quickly press the Superout button.

2.4.4 Collect the first 150 mL of effluent into the conical tube labeled "W1" (waste) and 12 X 25 mL fractions into the numbered conical tubes each pre-filled with 225 mL CMRL 1066, Supplemented, as described on the Purification Data Log for each respective COBE run.

2.4.5 Once the fractions are collected, stop the COBE and discard the COBE bag and tubing.

2.4.6 To evaluate each COBE fraction quickly, gently but thoroughly mix each fraction from step 9.1.3.4, then quickly transfer a 0.5 mL sample to one well of a 12-well microtiter plate and 0.5 mL of the W fraction to 35 mm dish.

2.4.7 Stain each sample with dithizone according to the institution's procedure and observe for islets. Record observations on the Re-purification Data Log.

2.4.8 Centrifuge the 250 mL tubes for 3 minutes at 140 x g and 2ºC to 8ºC. Record Packed Tissue Volumes of each COBE fraction on the Re-purification Data Log. Discard the supernatant.

Note: Scoring Guidelines for purified layers in Purification Data Logs:

- Packed Tissue Volume: estimate of the tissue volume in the individual conical tubes after they have centrifuged for 3 minutes at 140 x g and 2ºC to 8ºC.
- % Purity: estimate relative amount (%) of islets to total tissue.
- H M L D: This is the disposition for each conical tube as defined in the column header.

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Supplementary Purification, OptiPrep Procedure, Data Log

Layer	Medium	Amount
Capping Layer	CIT Cold Storage Solution	30 mL
Tissue Layer	_____ mL packed tissue in this COBE Run, plus 20 mL of Albumin Human USP, 25% Solution, and q.s. to 120 g with CIT Cold Storage Solution	120 g
Density Gradients	Low Density Gradient (1.06 g/mL)	125 g
	High Density Gradient (1.10 g/mL)	125 g
Bottom	High Density Gradient (1.10 g/mL)	120 g

Centrifuge Start Time		**Centrifuge Stop Time**	

#	CMRL 1066, Supplemented Pre-fill Vol. (mL)	Fraction Volume Collected (mL)	Packed Tissue Volume (mL)	Comments	Islet Purity (%)	Disposition: H: High, M: Middle, L: Low, D: Discard (Circle One)
W	0	150				H M L D
1	225	25				H M L D
2	225	25				H M L D
3	225	25				H M L D
4	225	25				H M L D
5	225	25				H M L D
6	225	25				H M L D
7	225	25				H M L D
8	225	25				H M L D
9	225	25				H M L D
10	225	25				H M L D
11	225	25				H M L D
12	225	25				H M L D
Bag	0	95				D

Comments on supplementary purification:_____

_____

_____

Recorded by: _____ **Date:** _____

Verified by: _____ **Date:** _____

Islets Lot Number: _____

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2.5 Combine fractions with purity of 30% or greater with the complimentary fractions from Section 8.3.10, and record the disposition of each fraction in the Supplementary Purification OptiPrep Procedure, Data Log, Section 2.4.8, above. Discard fractions < 30% pure. Keep the conical tubes flat on the bench at room temperature until the tissue of all COBE runs has been combined into the respective conical tubes.

Performed by: _____ **Date:** _____

Verified by: _____ **Date:** _____

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