Purified Human Pancreatic Islets, Supplementary Purification, Discontinuous Polysucrose Procedure & Record – *A Standard Operating Procedure of the NIH Clinical Islet Transplantation Consortium*

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Document Title:

PURIFIED HUMAN PANCREATIC ISLETS SUPPLEMENTARY PURIFICATION, DISCONTINUOUS POLYSUCROSE PROCEDURE & RECORD

1.0 MATERIALS

Material	Source	Lot #	Expiration Date	Quantity Required	Quantity Used
Cold Storage/ Purification Stock Solution				500 mL	mL
Stock Polysucrose Solution, sterile 1.132 g/mL	Mediatech Product No. 99-662-CVS			350 mL	
Islet Gradient 1.108 g/mL	Mediatech Product No. 99-692-CIS			75 mL	mL
Islet Gradient 1.096 g/mL	Mediatech Product No. 99-691-CIS			75 mL	mL
Islet Gradient 1.037 g/mL	Mediatech Product No. 99-690-CIS			75 mL	mL

2.0 PROCEDURE

2.1 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.

- Place the tubing into the valve slots on the COBE (but outside the pinch valves), using the color-coding to determine position. The tubing is not loaded into the pinch valves, but merely held in place in the tubing guides.
- Clamp yellow, purple, and blue tubing using tubing clamps or heat sealer.
- Red and green tubing remains opened or unclamped.
- Connect one end of 36 inch tubing (#16) to the COBE tubing (pink color) and the other end remains in the 250 mL conical.
- Prepare the COBE according to the institution's procedures.

Verified by: _____ **Date:** _____

2.2 Islet Washing

2.2.1 Transfer the Rescue Islets (up to 40 mL of packed tissue volume) from PBR Section 8.5 to a 250 mL conical tube, fill the tube with cold CIT Cold Storage/Purification Stock Solution, centrifuge it at 2 to 8ºC and 140 X gravity for three minutes. Remove the supernatant solution, making sure that the pellet is visible at all times.

Islets Lot Number: _____

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2.2.2 Fill the conical tube again with cold CIT Cold Storage/Purification Stock Solution and re-suspend the islets with a gentle swirling motion. Seal the tube and place it in a refrigerator at 2 to 8ºC for 30 to 50 minutes or proceed to the purification immediately.

Verified by: _____ **Date:** _____

2.3 Rescue Gradient Islet Purification

2.3.1 Remove the Rescue Islets (Section 9.2.2, above) conical tube from the refrigerator. Centrifuge at 2 to 8ºC and 140 X g for 3 minutes. Remove the supernatant, and gently re-suspend the tissue in 1.132 g/mL density gradient to a volume of 250 mL. Load the tissue suspended in 1.132 g/mL density gradient into the COBE bag. Rinse the conical tube with 50 mL of 1.132 g/mL gradient and load the rinse into the COBE bag.

2.3.2 While the suspension is flowing into the COBE bag, add 125 mL of CIT Culture Media to each of four 250 mL conical tubes.

2.3.3 After all the suspension has entered the COBE bag clamp the tubing. Load 1.108 g/mL gradient with the peristaltic pump through this tubing up to the T-junction. When the solution reaches the T-junction, turn the pump off, and clamp the tubing.

2.3.4 Remove the air in the COBE bag as follows:

- Unclamp the tubing to the transfer bag.
- Set the COBE speed to 2,000 rpm.
- Push the "START" button.
- When the centrifuge reaches 2,000 rpm, push the "SUPEROUT" button. Immediately adjust the SUPEROUT rate to 150 mL/minute. Solution will be pushed up through the tubing towards the T-junction.
- Clamp the tubing connected to the transfer bag as solution reaches the T-junction, and simultaneously press the "STOP/RESET" button.

2.3.5 When the COBE centrifuge stops, set the SUPEROUT rate dial to "0" and press the "START" button.

2.3.6 When the COBE centrifuge reaches 2,000 rpm, unclamp the tubing connected to the silicone tubing.

- Aseptically place the end of the silicone tubing in the bottle containing the 1.108 g/mL density gradient and start the pump at 90 mL/minute. Pump in 75 mL of 1.108 g/mL density gradient on top of the 300 mL of tissue suspension in the COBE bag. Stop the pump.
- Place the end of the silicone tubing in the bottle containing the 1.096 g/mL density gradient. Restart the pump and load 75 mL of 1.096 g/mL density gradient into the COBE bag. Stop the pump.
- Place the end of the silicone tubing in the bottle containing the 1.037 g/mL density gradient. Restart the pump and load 75 mL of 1.037 g/mL density gradient into the COBE bag. Stop the pump.
- Place the end of the silicone tubing in the bottle containing 50 mL of CIT Cold Storage Solution. Restart the pump and load this solution until the fluid/air interface reaches midway down the tubing leading to the rotation seal. Stop the pump.

Verified by: _____ **Date:** _____

Islets Lot Number: _____

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2.3.7 After the rotating seal is rinsed with CIT cold Storage Solution, turn off the pump, clamp the COBE set tubing connected to the silicone tubing, adjust the SUPEROUT valve to "0," open the pump head, and release the tubing from the pump head. Press the "SUPEROUT" button, immediately open a tube to release the excess pressure, and then re-clamp the tube. Allow the COBE centrifuge to spin for three minutes at 2000 rpm.

2.3.8 Collect four fractions:

- Collect the first fraction of 100 mL in the first conical tube (Tube 1). Press the "HOLD" button, move the tubing to the second labeled conical tube and
- Collect the next fraction of 75 mL in the second conical tube (Tube 2). Press the "HOLD" button, move the tubing to the third labeled conical tube and release the "HOLD" button.
- Collect the next fraction of 75 mL in the third conical tube (Tube 3). Press the "HOLD" button, move the tubing to the fourth labeled conical tube and release the "HOLD" button.
- Collect the next fraction of 100 mL in the fourth conical tube (Tube 4). Press "Stop" button.

Verified by: _____ **Date:** _____

2.3.9 Re-suspend the tissue in tubes 1 – 4 from the Rescue Purification Run to a total weight of 100 g or 100 mL each by adding CIT Culture Media, where necessary.

2.3.10 Mix with a gentle swirling motion, and aseptically take up to 0.5 mL samples of each of the 4 fractions. Each 0.5 mL sample is placed in one well of a 12-well microtiter plate.

2.3.11 Stain each sample with dithizone and observe for islets. Record observations on the table, below.

Note: Evaluation Guidelines for Rescued Islets Fractions, below.

- Pellet Volume: this is an estimate of the tissue volume in the individual conical tubes after they have been centrifuged.
- % Purity: estimate relative amount (%) of islets to total tissue.
- H M L R D: This is the disposition for each conical according to the column header.

Supplementary Purification, Discontinuous Polysucrose Procedure, Data Log

Tube #	Volume Collected (mL)	Pellet Volume (mL)	Comments	Islet Purity (%)	Disposition: H: High, M: Middle, L: Low, D: Discard (Circle One)
1					H M L D
2					H M L D
3					H M L D
4					H M L D

Performed by: _____ **Date:** _____

Verified by: _____ **Date:** _____

Islets Lot Number: _____

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2.3.12 Centrifuge the tubes 1 – 4 at 2 to 8ºC and 140 X g for 3 minutes. Record their packed tissue volumes in the table in Section 9.3.12, above.

2.3.13 Based on the data in the table in Section 9.3.12, above, discard as bio-hazardous waste any tube with islets purity less than 30%.

2.3.14 High Purity Islet Washing

Add 250 mL of CIT Culture Media to the tube containing High Purity Islets and centrifuge it at 2 to 8ºC and 140 X g for 3 minutes with the brake on. Remove the supernatant solution, making sure that the pellet is visible at all times. Repeat this wash a second and a third time with CIT Culture Media.

2.3.15 Middle Purity Islet Washing

Add 250 mL of CIT Culture Media to the tube containing Middle Purity Islets and centrifuge it at 2 to 8ºC and 140 X g for 3 minutes with the brake on. Remove the supernatant solution, making sure that the pellet is visible at all times. Repeat this wash a second and a third time with CIT Culture Media.

2.3.16 Low Purity Islet Washing

Add 250 mL of CIT Culture Media to the tube containing Low Purity Islets and centrifuge it at 2 to 8ºC and 140 X g for 3 minutes with the brake on. Remove the supernatant solution, making sure that the pellet is visible at all times. Repeat this wash a second and a third time with CIT Culture Media.

2.3.17 Combine fractions with islet purity of 30% or greater with the complimentary fractions from PBR Section 8.3.10 and record the disposition of each fraction in the table in the Supplementary Purification, Discontinuous Polysucrose Procedure, Data Log, Section 2.3.12, 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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