Purified Human Pancreatic Islets, Supplementary Purification, Biocoll 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, CONTINUOUS BIOCOLL PROCEDURE & RECORD

1.0 MATERIALS:

Material	Source	Lot #	Expiration Date	Quantity Required	Quantity Used
Viaspan (UW Solution)				314.3 mL	mL
Biocoll Separating Solution, Density 1.100	Biochrome AG/ Cedarlane			105.7 mL	mL

2.0 PROCEDURE:

2.1 Prepare the tissue by adding 150 mL of UW Solution to the Supplementary Purification Islets from PBR Section 8.5.

Note: When using this Biocoll Supplementary Purification procedure, up to 45 mL of packed tissue volume can be loaded on the COBE for each run. It is very important not to overload the COBE.

Note: The volume of UW Solution for each run remains constant, regardless of the volume of the packed tissue.

Volume of UW Solution used for each COBE run: _____ mL

Total Packed Tissue Volume: _____ mL

Number of COBE runs: _____

Packed Tissue Volume prepared for each COBE run: _____ mL

Performed by: _____ **Date:** _____

2.2 Incubate the tissue in UW solution for 30 minutes on ice or in the cold room, using the Maxi-rotator (or mix the tissue in the tube by swirling every 5 minutes).

Performed by: _____ **Date:** _____

2.3 Preparation of Biocoll Heavy (49% Biocoll/51% UW Solution mixed) and Light (30% Biocoll/70% UW Solution mixed) density gradients:

2.3.1 Pipette 66.3 mL of UW Solution into one sterile bottle. Label this Bottle with "**Heavy Gradient**," Islets Lot Number, date and time of preparation, and initials of preparer.

2.3.2 Pipette 98.0 mL of UW Solution into another sterile bottle. Label this Bottle with "**Light Gradient**," Islets Lot Number, date and time of preparation, and initials of preparer.

Islets Lot Number: _____

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2.3.3 Pipette 63.7 mL of 1.10 g/mL Biocoll Gradient Solution into the bottle labeled "**Heavy Gradient**" and quickly swirl bottle to mix properly.

2.3.4 Pipette 42.0 mL of 1.10 g/mL Biocoll Gradient Solution into the bottle labeled "**Light Gradient**" and quickly swirl bottle to mix.

Performed by: _____ **Date:** _____

2.4 Set the COBE at 1500 rpm and Superout at 0. Press Start to start the COBE.

2.5 Add 110 mL of 1.10 g/mL Biocoll Gradient Solution to the first (front) beaker and start the peristaltic pump on the maximum setting.

2.6 After all the Biocoll Gradient Solution is loaded onto the COBE, press Superout, turn off the pump, unclamp the pump head, and turn Superout to 100.

2.7 When the Biocoll Gradient Solution reaches the beaker, quickly re-clamp the pump head. Stop the COBE and turn Superout back to 0. Change the COBE speed to 3,000 rpm. All air should now be out of the system.

2.8 Add 130 mL of Heavy Gradient to the front beaker. Unclamp the line between the beakers briefly and re-clamp to get all air out.

2.9 Add 140 mL of Light Gradient to the second (rear) beaker.

2.10 Turn the pump speed down to 20 mL/min on the peristaltic pump and turn magnetic stirrer on the lowest setting. Start the COBE. Start pump. Unclamp the line between the beakers.

2.11 When nearly all the Biocoll is loaded onto the COBE, tilt the magnetic stirrer forward to ensure all Biocoll is loaded. Before the last bit of Ficoll is loaded, stop the stirrer and begin to slowly add the suspended islets to the front beaker.

2.12 When all tissue has been added, rinse the conical which contained the suspended islets with 50 mL of HBSS, 1X, and add this volume to the front beaker.

2.13 When everything has been loaded onto the COBE, clamp the tubing above the bag, press Super-Out (set at 0), turn off the pump and unclamp the pump head.

2.14 SLOWLY, unclamp the clamp above the COBE bag and start the timer.

Performed by: _____ **Date:** _____

2.15 Centrifuge for 5 minutes.

2.16 Prepare collection rod and line for fraction collection.

2.17 Prepare 12 X 250 mL conical tubes. Label them #1 through #12. Leave Tube #1 empty, and pre-fill Tubes #2 through #12 with 220 mL each of CMRL 1066, Supplemented.

Performed by: _____ **Date:** _____

Islets Lot Number: _____

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2.18 After 5 minutes, slowly adjust the Superout up to 100 and begin collecting tissue into the conical tubes.

2.19 Collect 150 mL of effluent in Tube #1. Collect 30 mL of effluent in Tubes #2 through #12, to a total volume of 250 mL in each tube.

2.20 When all effluent has been collected, press Stop on the COBE.

Performed by: _____ **Date:** _____

2.21 To evaluate each COBE fraction quickly, gently but thoroughly mix each fraction from Section 9.2.19, then quickly transfer a 0.5 mL sample to one well of a 12-well microtiter plate.

2.22 Stain each sample with dithizone according to the institution's procedure and observe for islets. Record observations on the Biocoll Supplementary Purification Data Log for each COBE run, below.

2.23 Centrifuge the 250 mL tubes for 3 minutes at 140 X g and 2ºC to 8ºC. Record the Packed Tissue Volumes of each COBE fraction on the Biocoll Supplementary Purification Data Log for each respective COBE run. Discard 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, Continuous Biocoll Procedure, Data Log, COBE Run #1:

#	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)
1	0	150				H M L D
2	220	30				H M L D
3	220	30				H M L D
4	220	30				H M L D
5	220	30				H M L D
6	220	30				H M L D
7	220	30				H M L D
8	220	30				H M L D
9	220	30				H M L D
10	220	30				H M L D
11	220	30				H M L D
12	220	30				H M L D
Centrifuge Start Time				**Centrifuge Stop Time**		

Comments on purification: _____

_____

_____

_____

Recorded by: _____ **Date:** _____

Verified by: _____ **Date:** _____

Islets Lot Number: _____

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2.24 Repeat all steps for each COBE run.

Supplementary Purification, Continuous Biocoll Procedure, Data Log, COBE Run #2:

#	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)
1	0	150				H M L D
2	220	30				H M L D
3	220	30				H M L D
4	220	30				H M L D
5	220	30				H M L D
6	220	30				H M L D
7	220	30				H M L D
8	220	30				H M L D
9	220	30				H M L D
10	220	30				H M L D
11	220	30				H M L D
12	220	30				H M L D
Centrifuge Start Time				**Centrifuge Stop Time**		

Comments on purification: _____

_____

_____

Recorded by: _____ **Date:** _____

Verified by: _____ **Date:** _____

2.25 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, Continuous Biocoll Procedure, Data Logs in Sections 2.23 and 2.24, 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:** _____

Islets Lot Number: _____

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