STANDARD OPERATING PROCEDURE (SOP)

Packaging and Shipping of Flash Frozen Non-Islet Pancreatic (Acinar) Tissue

Version: SHP-007-02
Standard Operating Procedure for Packaging and Shipping of Flash Frozen Non-Islet Pancreatic (Acinar) Tissue

SOP #: SHP-007-02

Version: 02
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Details:
Standardized Packaging and Shipping of Flash Frozen Non-Islet Pancreatic (Acinar) Tissue for IIDP Distribution based on the Prodo Labs, Inc. Protocols

Page Number: Page 2 of 9
1.0 Objective

1.1 To define a standardized method for packaging and cold shipping of research quality non-islet pancreatic (acinar) tissue (NIPT) to approved investigators for use in the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) sponsored research in the Integrated Islet Distribution Program (IIDP). This will also include reference to flash frozen NIPT tissue.

Note: This SOP was developed based on the Prodo Labs, Inc. shipping protocol and results from preliminary studies conducted by the IIDP and commissioned by the original IIDP Project Officer, and External Evaluation Committee (EEC).

2.0 Scope and Applicability

2.1 This SOP applies to all IIDP islet distribution centers using funds from the NIDDK to manufacture purified human NIPT for basic research studies for IIDP approved investigators.

2.2 This SOP will require participation from all participating IIDP centers.

3.0 Responsibilities

3.1 It is the responsibility of each IIDP center to follow the procedures listed in this SOP and to work to the best of their abilities to follow all requirements.

3.2 Managers and supervisors are responsible for assuring that all technicians are properly trained in the correct procedure for this SOP and that equipment and facilities are in good working order.

3.3 Laboratory personnel are responsible for reading and understanding the SOP and for performing the tasks in accordance with this SOP.

3.4 It is the responsibility of the IIDP CC to ensure adherence to the procedures outlined in this SOP. In order to accomplish this, the IIDP CC will interact with the relevant personnel from each of the participating centers.

3.4.1 The CC is responsible for the provision of the specified supplies listed in this protocol to the distributing centers.
3.4.2 The CC is responsible for the education of the investigators as to the advantages of this shipping method based on results of the preliminary studies.

3.5 It is the responsibility of each approved investigator receiving NIPT through the IIDP to cooperate in providing feedback to the IIDP on the condition of the NIPT upon receipt and to reuse and recycle all shipping materials to the best of their ability.

4.0 Definitions

4.1 **Integrated Islet Distribution Program (IIDP):** The IIDP is a program commissioned and funded by the NIDDK to provide quality human islets and NIPT to the diabetes research community to advance scientific discoveries and translational medicine. The IIDP consists of the NIDDK Project Scientist and Program Official, the External Evaluation Committee and the CC at City of Hope (COH). The IIDP CC integrates an interactive group of academic laboratories including the subcontracted IIDP centers.

4.2 **IIDP Coordinating Center (CC):** Joyce Niland, Ph.D., IIDP Principal Investigator leads CC staff to coordinate the activities of the IIDP and assists the participating centers and investigators in the distribution of human islets and NIPT.

4.3 **Approved Investigators:** Researchers who have requested NIPT from the IIDP for basic science studies and whose research protocols have been reviewed and approved by the IIDP.

4.4 **Non-Islet Pancreatic (Acinar) Tissue:** During a standard human islet isolation, the digested tissue goes through a purification process that allows the majority of the isolated islets to be separated from the acinar and other pancreatic tissues. This is commonly done using density gradients and a COBE centrifugation processor. The lighter density tissues, including the majority of human islets, are collected from the slurry, leaving the remaining pancreatic tissue, in the COBE processor bag that still holds the denser gradients and tissue. This non-islet pancreatic tissue is commonly referred to as the “Acinar” tissue, although it contains other types of pancreatic tissue, often including imbedded islets, ductal tissue, and blood vessel fragments.

5.0 Materials

5.1 The IIDP will provide each center with the following supplies necessary for shipments:
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<table>
<thead>
<tr>
<th>Vendor Name</th>
<th>Item #</th>
<th>Item Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fisher</td>
<td>MT61277RG(F)</td>
<td>Ciprofloxacin hydrochloride – 5 gm bottle (Corning 61277RG) / 1gm bottle (61277RF)</td>
</tr>
<tr>
<td>Fisher</td>
<td>501784544</td>
<td>Trypsin Inhibitor from Glycine max (soybean) – lyophilized powder</td>
</tr>
<tr>
<td>Fisher</td>
<td>5000-0050</td>
<td>Nalgene; Sterile 5.0 mL External Threaded Cryogenic Vials with Conical Bottom</td>
</tr>
<tr>
<td>Uline</td>
<td>S-5045</td>
<td>8 x 11” Labels for Outside of boxes</td>
</tr>
<tr>
<td>Prodo Labs</td>
<td>PIM-G</td>
<td>5mL –Glutamine/Glutathione</td>
</tr>
<tr>
<td>Prodo Labs</td>
<td>PIM-T</td>
<td>500mL – Transport Media</td>
</tr>
</tbody>
</table>

5.2 Hanks Buffered Salt Solution (HBSS) – no additives

5.3 15mL, 50mL and 250mL conical tubes (depending on final volume of NIPT requested).

5.5 Cryogenic markers or pencils for tube labeling.

5.6 Centrifuge with capabilities of spinning the appropriate sized conicals and the 5.0 mL cryogenic vials at 1000 rpm for 2 minutes.

5.7 Sterile pipettes, pipettors, and other appropriate sterile lab ware for manipulation of islet preparations.

5.9 Federal Ex Shipping Forms.

5.10 Liquid nitrogen and appropriate vessels and equipment for safely freezing the NIPT.

5.11 Appropriate sized Styrofoam shipping boxes and adequate dry ice.

6.0 Procedures

6.1 Receipt of Supplies
   The majority of supplies should be stored in appropriate dry, temperature-controlled environments (room temperature 16°-28°C).

6.1.1 The Prodo Labs PIM-T should be stored between 2° and 8°C upon receipt but is stable at room temperature.

6.1.2 The Gemini AB serum and the PIM-G vials should be stored at -5° to -20°C.
6.1.3 The Ciprofloxacin can be stored on the shelf but filter sterilized suspension aliquots should be stored at -5° to -20°C.

6.1.4 The T9128 Trypsin Inhibitor from Glycine max (soybean) – lyophilized powder should be stored between 2° and 8°C upon receipt.

6.2 Begin NIPT (Acinar) Broadcast

6.2.1 Begin Acinar broadcast on IIDP Secure Access Center Website as soon as you are sure that an islet isolation will be taking place.

6.2.2 Enter donor and isolation information on IIDP secure website under Quick Acinar Broadcast. Complete Donor Information Form and Processing and Distribution of Acinar Tissue Form.

6.2.3 Donor Information Form is same as for islets and all entered information will be auto-populated to the subsequent Islet Broadcast.

6.2.4 Relevant information entered on the Processing and Distribution of Acinar Tissue Form will be auto-populated to the Islet Broadcast.

6.2.5 Complete information regarding NIPT will be available as freshly isolated (SOP-SHP-006) and/or flash frozen (SOP-SHP-007).

6.2.6 Complete the broadcast records and proceed to Broadcast.

Note: The Broadcast Deadline should be set for the approximate time of when the purification will be completed so that the total amount of accepted tissue will be known prior to when the NIPT tissue is to be prepared. Freshly isolated NIPT should be sent out on the day of the isolation or at the next available FedEx pickup, within 24 hours of isolation (SHP-006). This will limit freshly isolated NIPT broadcasts to Sunday evenings through Thursdays (or Fridays if investigators will accept Saturday deliveries). The NIPT Broadcasts are done prior to the Islet Broadcasts. However, Flash Frozen NIPT (SHP-007) can always be offered, no matter the day or isolation, as tissue can be held until a mutually acceptable shipping date can be agreed upon. It should be held at -20°C and shipped on dry ice by FedEx overnight shipment.

6.2.7 Set your NIPT Broadcast Deadline to the assumed time when the purification will be completed. This will ensure that the NIPT tissue will have minimum holding
time prior to freezing the requested NIPT or packaging of the freshly isolated samples. (See SOP SHP-006)

6.2.8 When Deadline of Broadcast is met, an automated email will be sent to center asking to View Acinar Offers and to confirm the shipment or cancellation of each request.

6.3 Collection of NIPT for Transport

6.3.1 The NIPT, the tissue remaining in the COBE purification bag, should be transferred under sterile conditions, into two 250 mL conical tubes, conical tubes, as soon as feasible during the islet isolation process. Top with Hanks Buffered Salt Solution (HBSS), cap, invert to mix and dilute Ficoll, and centrifuge at 4°C for 2 minutes @180 g.

6.3.2 Prepare PIM-T media prior to the collection by thawing and adding 5mL of PIM-G, 12.5mL of AB serum (2.5% v/v), and 0.5mL of prepared sterile ciprofloxacin stock aliquot per SHP-006 Attachment 8.3 to 500 mL of PIM-T to make PIM-T Complete.

6.3.3 Once deadline has been reached and final amounts of accepted NIPT is confirmed, calculate the amount of PIM-T Complete that is needed for all shipments. Add 1 mL of Trypsin Inhibitor stock (Attachment 8.4) to each 100 mL of PIM-T Complete for NIPT shipping (~150mL of media/1mL NIPT total requested). This will give a final working concentration of 100μg/mL of Trypsin inhibitor in the media. Resuspend only the amount of NIPT tissue needed to complete the requests in 10x the volume of PIM-T Complete plus inhibitor.

6.3.4 If deadline has not yet been reached and the total amounts of NIPT accepted is not yet known, suspend up to 20 mL of pellet to 10 times the pellet volume with PIM-T Complete plus inhibitor (200mL). Lay conical of the NIPT suspension on its side at 4°C to avoid pelleting of NIPT while waiting to confirm acinar shipments. Note: As program expands more tissue may be held as recipient requests increase.

6.3.5 Note: Once inhibitor is added to the PIM-T Complete, it must be used on the day of addition.
6.4 Preparation of Flash Frozen NIPT

6.4.1 As investigator’s requests are determined through the broadcast system, prepare appropriate amounts of NIPT into proper sized consolidation vessels for aliquoting and freezing from the diluted samples in in 6.3.3/6.3.4 above. Label each conical for identification during transfer. Label corresponding 5mL cryo tube with RRID #, date, amount of requested Acinar tissue and recipient name and hold until ready for final transfer.

6.4.1.1 For 0.5 – 1.0mL of requested NIPT pelleted tissue, add 5 - 10mL of NIPT suspension to 15mL conical.

6.4.1.2 For 1.1 – 2.0mL of requested NIPT pelleted tissue, add 11-20mL of NIPT suspension to 50mL conical.

6.4.1.3 For 2.1 – 3.0mL of requested NIPT pelleted tissue, add 21-30 mL of NIPT suspension to 50mL conical.

6.4.1.4 For 3.1 – 4.0mL of requested NIPT pelleted tissue, add 31-40mL of NIPT suspension to 50mL conical.

6.4.2 Centrifuge all samples at 4°C for 2 minutes @180 g to pellet the acinar tissue. Remove the supernatant and wash the tissue with HBSS without serum by topping each conical with appropriate amount of HBSS. This will remove the serum (protein) from the acinar tissue. Resuspend tissue. Repeat the centrifugation.

6.4.3 Remove supernatant. Wet pipet and transfer each pellet to corresponding labeled 5 mL Cryo tube, rinsing original tube with HBBS without serum to insure all tissue is transferred. Centrifuge cryo tubes at 4°C for 2 minutes @180 g to pellet the acinar tissue. Remove all supernatant to ensure a dry, serum-free pellet.

6.4.4 Freeze pellet by placing tube in liquid nitrogen per your center’s protocol. Store at -80°C until mutual time for shipping is agreed upon by center and investigator to assure no problems with receipt.

6.4.5 When it is time to ship the flash frozen NIPT, place sample in a small, thick Styrofoam shipping box filled with approximately five pounds of dry ice. Label with IIDP frozen tissue label. Ship using FedEx overnight shipment.
6.5 Completing the Distribution Records

6.5.1 The shipping center will enter the NIPT to be shipped (Confirm Recipient screen) and will enter FedEx tracking numbers Broadcast System. In addition, the shipping center should process the shipment by using the on-line FedEx process and click the prompt to alert the recipient of the FedEx tracking number. This will help the recipient investigator follow their shipment through the FedEx system and free up time for the centers and the IIDP.

*Note: If the investigator has picked up the NIPT at the distributing center then the center should check “Yes” in the “Direct Pickup” column and “Yes” under “NIPT Shipped?” once they are picked up.*

6.5.2 The investigators will have the FedEx tracking numbers listed in their History of Acinar Tissue Offers table.

7.0 References

7.1 Prodo Labs Shipping Protocol VS Standard IIDP Shipping SOP Pilot Study Design, Protocols, Feedback, Analysis, and Center Comments

7.2 Tip Temperature Products website: [http://www.tiptemp.com](http://www.tiptemp.com) and product inserts

7.3 Prodo Labs, Inc. Protocols and Website: [http://www.prodolabs.com](http://www.prodolabs.com)

8.0 Attachments

8.1 Ciprofloxacin Stock Preparation V2

8.2 Trypsin Inhibitor Stock Preparation