STANDARD OPERATING PROCEDURE (SOP)

Islet Culture and Preparation for Cold Shipping

Version: SHP-004-001
Standard Operating Procedure for Islet Culture and Preparation for Cold Shipping

SOP #: SHP-004-001

Version: 001
Supercedes: Draft
Issue Date: 11/10/14
Effective Date: 11/10/14

Details:
Standardized Islet Culture and Preparation for Cold Shipping of Human Islets for IIDP Distribution based on the Prodo Labs, Inc. Protocols

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1.0 Objective

1.1 To define a standardized method for post purification islet culture and maintenance in preparation for cold shipping of research quality islets 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).

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). It was commissioned due to problems with acquiring the supplies that were used in SHP-001 and with the hope that this method is a better means for transportation of IIDP islets. Preliminary studies proved the islets by the Prodo Labs’ method was statistically as good as the original IIDP method, it was preferred by the test researchers, and was much more cost effective for the IIDP. This new method may be modified as future methods are tested and approved by the Project Scientist (PS), the Program Official (PO), and 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 pancreatic islets for basic research studies for IIDP approved investigators.

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

2.2.1 The IIDP Coordinating Center (CC) acknowledges that the IIDP centers will transition into this protocol as training is accomplished, supplies are received, and all concerns of special research projects have been met.

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 media and their additives listed in this protocol to the distributing centers.

3.4.2 The CC will work with the Prodo Labs, Inc. staff to properly train the staff of all IIDP centers in the techniques and nuances of this protocol in order to make the transition as easy as possible for the IIDP centers.

3.4.3 The CC is responsible for the education of the investigators as to the advantages of this culture method based on results of the preliminary studies and past data of longer culture times collected by the CC.

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 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 IIDP CC at City of Hope (COH). The 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.

5.0 Materials

5.1 The IIDP will provide each center with the following supplies necessary for islet culture:

<table>
<thead>
<tr>
<th>Company/Manufacturer Name</th>
<th>Order#</th>
<th>Item Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prodo Labs, Inc.</td>
<td>PIM-G001GMP</td>
<td>5mL – Glutamine/Glutathione</td>
</tr>
<tr>
<td>Prodo Labs</td>
<td>PIM-R001GMP</td>
<td>500mL – Recovery Media</td>
</tr>
<tr>
<td>Gemini Bio Products</td>
<td>100512</td>
<td>100mL - Human AB Serum (ABS)HI- LOT # H15M03A</td>
</tr>
<tr>
<td>Cellgro, Inc.</td>
<td>61-277RG</td>
<td>Ciprofloxacin hydrochloride – 5 gm bottle</td>
</tr>
</tbody>
</table>
5.2 Supplies provided by the IIDP Centers:

5.2.1 Islets for distribution.

5.2.2 Routine lab supplies for transferring, media changing and counting islets.

6.0 Procedures

6.1 Receipt of Supplies

6.1.1 The Prodo Labs PIM-R 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.

6.2 Post Purification Culture of Islet with ≥70% Purity

6.2.1 Prepare 1 bottle of PIM-R media prior to the isolation by thawing and adding 5mL of PIM-G, 25mL of AB serum (5% v/v), and 0.5mL of prepared ciprofloxacin sterile aliquot per SHP-004 Attachment 8.1. (If culture for IIDP distribution is greater than 250,000IEQ, a second bottle of PIM-R will need to be prepared.

6.2.1.1 (Note: If a prepared media bottle is to be used from a previous isolation, it must have been filter sterilized at the end of the previous use.)

6.2.2 Post purification, all pooled islets with > 70% purity should be brought up in 200mL of PIM-R in a 250mL conical. Thoroughly mix suspension by either pouring between 2 conicals or inverting one conical at least 3 times. Quickly removing cap and count samples should be taken to the exact volume required by a second technician. (This can be accomplished using center specific method as long as a representative sample is taken from a well mixed suspension and the sample is suspended in PIM-R.)

6.2.2.1 While counting, place the conical that contains the islets on its side at room temperature in the hood so that the islets do not pellet.
6.2.3 Once the average count has been determined from the samples taken and recorded on the count sheets, calculate the total IEQs and the IEQ per mL in the islet suspension.

6.2.3.1 Example: If 250,000IEQ were in the 200mL suspension, then 250,000IEQ / 200mL = 1,250IEQ/mL.

6.2.4 Calculate the amount of suspension needed to aliquot 20,000IEQ per flask.

6.2.4.1 Example: 20,000IEQ/1,250IEQ/mL = 16mL/Flask

6.2.5 Calculate the number of flasks needed to culture the islets for broadcast at 20,000 IEQ/Flask.

6.2.5.1 Example: 250,000IEQ / 20,000IEQ = 12.5 flasks (or 200mL /16mL= 12.5.) Round up the fractional number of flasks to the next whole number and recalculate the proper aliquot to be taken from the islet suspension.

6.2.5.2 Example: 12.5→13 Flasks; 200mL /13 flasks = 15.4mL islet suspension/flask.

6.2.5.3 Determine the amount of media needed to prewet each flask. Example: 40mLs - 15.4mL islets =24.6mL fresh PIM-R.

6.2.6 Aseptically transfer the proper amount of T-175 non-coated flasks into the hood and label per center protocol. Pre-wet all but one flask with predetermined amount of fresh PIM-R.

6.2.6.1 For the final flask, pre-wet with 6mL less than the calculated amount and mark as final flask.

6.2.7 Lay the flasks flat on the hood surface making sure the media covers the entire surface being careful not to wet the neck or cap of the flask.

6.2.8 Deliver ~20,000IEQ to appropriate flasks with a 25mL pipette properly mixing the islet containing solution between each flask to ensure even distribution.

6.2.9 Rinse the conical after the (marked) final flask has been loaded with 3mL of extra media. Repeat.
6.2.10 After culturing the islets, place all of the flasks in the incubator and set at 37°C±0.5 and 5% ±0.5 CO₂ and culture for at least 48 hours.

   Note: See SHP-005 Attachment 8.1 for possible exceptions for culture time.

6.2.11 Between 12 and 18 hours after culture begins, perform a 50% media change on all flasks.

6.3 Post Purification Culture of Islet with <70% Purity

6.3.1 Follow all steps outlined in 6.2 but determine the concentration of the cultured islets and the number of flasks needed in 6.2.5.1 by multiplying the total flasks by the percent purity.

6.3.1.1 Example: If part of the prep has a 50% purity and 50,000IEQ then 50,000IEQ / 20,000IEQ = 2.5 flasks x 50% purity = 5 flasks.

6.3.1.2 Determine all other values based on this calculation.

   Note: Islets with < 50% purity are not eligible for reimbursement.

6.4 Half Media Change Procedure

A Media change of half the culture volume should be performed 18 -24 hours after the completion of the isolation, Day 1 post culture. If for some reason the islets cannot be shipped out before or on Day 5, a second media change should be performed. (Note: It is unlikely that this scenario will occur.)

6.4.1 Warm up required amount of PIM-R to room temperature by taking it out of the refrigerator at least 1 hour before the media change is performed. Aseptically place into the laminar flow hood.

6.4.2 Remove the flask(s) from the incubator, keeping them vertical (with the cap facing upward) while transporting from incubator and aseptically place the flask(s) into laminar flow hood.

6.4.3 Rock the flasks gently back and forth keeping them horizontal, to get any islets that are loosely attached to the bottom of the flasks to go into the solution, being careful avoiding any media getting into the caps of the flasks. Return upright and loosen the caps of the flask(s).
6.4.4 Arrange a 50mL conical rack supported by a 250mL conical rack for each set of 10 flasks. Placing 5 in each row, position flask(s) at an angle, tilting it towards the longer edge using 250mL and 50mL conical racks for support, resting them on the longer edge of the 50mL conical rack so all islets can congregate to the lower corner of the flask due to gravity. Start a timer set for 45 minutes and record start time in the batch record.

6.4.5 Leave all flask(s) positioned in this manner for 45 minutes to allow islets to settle in the bottom corner surface.

6.4.6 Go to the middle point of the media in one flask and take a 1mL sample, place in a Petri dish, and examine under the microscope. If islets are less than 50 micron in size proceed to the next step. If larger islets are visible allow more settling time until the media sample has no islets present.

6.4.7 While maintaining the position of the flask (as in 6.4.5) aspirate 50% of the used media (20mL) from the surface of the liquid layer equidistant from the sides of the flask, without disturbing the islets that are settled in the bottom corner.

6.4.8 Pipette the aspirated used media into an empty sterile container. (This can be checked when the media change is completed for viable islets that can then be returned to a flask.) Repeat steps for all remaining flasks.

6.4.9 Pipette 20mL of fresh PIM-R into each flask after aspiration and tighten caps.

6.4.10 Once media change is completed for all the flask(s), check aspirate for any viable islets and transfer to a flask if any are found. Confirm all ventilated flask caps are tight and return to incubator set to 37° ±0.5°C.

6.4.11 Filter sterilize any media that is to be kept for future use if its bottle was entered during the preparation.

7.0 References

7.1 Prodo Labs, Inc. Media Preparation and Use Instructions

7.2 Prodo Labs, Inc. Culturing in PIM-R after Purification
7.3 Prodo Labs, Inc. Media Change Protocol

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

8.0 Attachments

8.1 Ciprofloxacin Stock Preparation