Jun 18, 2020

Islet Culture and Preparation for Cold Shipping

Integrated Islet Distribution Program[1]

[1][Integrated Islet Distribution Program, City of Hope, Duarte, CA]

1 Works for me dx.doi.org/10.17504/protocols.io.bfsfjnbn

Integrated Islet Distribution Program
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Integrated Islet Distribution Program
Integrated Islet Distribution Program, City of Hope

ABSTRACT

This SOP defines a standardized method for packaging and cold shipping of research quality islets to approved investigators of human isolated islet preparations, for use in the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) sponsored research in the Integrated Islet Distribution Program (IIDP). This protocol is written to assist the participating islet isolation centers and investigators who are part of this program.

Integrated Islet Distribution Program (IIDP) (RRID:SCR _014387)

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 IIDP SOP: 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 were 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 IIDP Team, Project Scientist (PS), the Program Official (PO), and EEC.

EXTERNAL LINK

https://iidp.coh.org/Investigators/Policies-Standard-Operating-Procedures

DOI

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PROTOCOL CITATION

Integrated Islet Distribution Program 2020. Islet Culture and Preparation for Cold Shipping. **protocols.io** dx.doi.org/10.17504/protocols.io.bfsfjnbn

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KEYWORDS

Human islets, Dithizone, Purity, Islet Equivalent, Actual Islet, Islet Quality Grade, Islet Index

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Apr 29, 2020

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36391

GUIDELINES

Responsibilities

- It is the responsibility of the IIDP CC to both follow and 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.
- It is the responsibility of each IIDP center to follow the procedures listed in this SOP and to work to the best of their ability to follow all requirements.

Definitions

- ***Integrated Islet Distribution Program (IIDP) (RRID:SCR_014387):*** The IIDP is a contracted 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, the Project Officer (PO), the External Evaluation Committee (EEC) and the Coordinating Center (CC) at City of Hope (COH). The IIDP CC integrates an interactive group of academic laboratories including the subcontracted IIDP centers.
- ***IIDP Coordinating Center (CC):*** Joyce Niland, Ph.D. is the Principal Investigator for the IIDP CC and leads staff from the Department of Diabetes and Cancer Discovery Science, Diabetes and Metabolism Research Institute at COH to coordinate the activities of the IIDP and assist the participating centers and investigators in the distribution of human islets.
- ***Islet Equivalent (IEQ)***: A conversion factor of the size of an actual islet to an equivalent size of an islet which is 150 µm diameter by mathematically compensating for the volume.
- ***Approved Investigators***: Researchers who have requested islets from the IIDP for basic science studies and whose research protocols have been reviewed and approved by the IIDP.
- ***Islet Allocation System (IA)***: This is the online system administered by the IIDP to allow fair distribution of basic science islets to approved investigators. This interactive system is used by the IIDP Centers and the approved investigators facilitates and tracks the distribution of human islets.

MATERIALS

NAME	CATALOG #	VENDOR
Dithizone (Diphenylthiocarbazone)	D5130	Millipore Sigma
Gibco DPBS without Calcium and Magnesium	14190136 or equivalent	Fisher Scientific
Dimethyl sulfoxide	D8779 or equivalent	Sigma Aldrich
Human AB Serum (ABS) HI	100-512; Heat Inactivated	Gemini Bioproducts
PIM(G)® (5 mL Glutamine/Glutathione)	PIM(G)®	Prodo Laboratories, Inc
Corning™ Ciprofloxacin Hydrochloride	MT61277RG (Corning™ 61277RG)	Fisher Scientific
PIM(R)®	PIM(R)®	Prodo Laboratories, Inc
MP Biomedicals™ Ciprofloxacin Hydrochloride or equivalent	MP219902005	Fisher Scientific

MATERIALS TEXT

Citation: Integrated Islet Distribution Program (06/18/2020). Islet Culture and Preparation for Cold Shipping. https://dx.doi.org/10.17504/protocols.io.bfsfjnbn

Gilson™ PIPETMAN Classic™ Pipets-F123601 or equivalent
Adjustable pipettor -P200

Gilson F123601G

50 to 200µL, ±0.5, ±1µL

Fisherbrand™ Large-Orifice Pipet Tips, 1 to 200µL or equivalent
Genomic/Wide Orifice Pipet Tips

Fisherbrand 02-707-134

1 to 200µL

Fisherbrand™ Petri Dishes with Clear Lid or equivalent
Petri Dishes

Fisherbrand FB0875713A

Round, Raised Ridge, 60mm, 15mm

Thermo Scientific™ Nalgene™ Rapid-Flow™ Sterile Disposable Bottle Top Filters with PES Membrane
0.2 µm Bottle Top Filter or equivalent

Nalgene 09-741-07

500 mL, 0.2 µm PES Bottle Top Filter

Citation: Integrated Islet Distribution Program (06/18/2020). Islet Culture and Preparation for Cold Shipping. https://dx.doi.org/10.17504/protocols.io.bfsfjnbn

Invitrogen™ EVOS™ XL Core Imaging System

Digital, transmitted light, inverted imaging system or equivalent

Invitrogen Microscope 12-562-751

EVOS XL Core Imaging System with fixed stage

Bal Supply Cell Counter or equivalent

Cell Counter (Manual or Electronic)

Bal Supply 02-670-14

Drummond™ Fixed-Volume Microdispensers or equivalent

Drummond 3000385

Drummond™ 21176F

Volumetric Range 100/200UL with borosilicate glass bores

Corning 4492 or equivalent

Sterile Polystyrene Serological Pipets

Corning™ Stripette™ Wide-Tip Disposable Polystyren

Sterile, individually wrapped, and certified nonpyrogenic and DNase-/RNase-free

Accuracy within ±2% at full volume

07-200-619

Citation: Integrated Islet Distribution Program (06/18/2020). Islet Culture and Preparation for Cold Shipping. https://dx.doi.org/10.17504/protocols.io.bfsfjnbn

Thermo Scientific™ Nunc™ Non-treated Flasks
T-175 Non-treated Flasks

Thermo Scientific™ Nunc™ 12-566-85
T-175 Non-treated Flasks

EQUIPMENT

NAME	CATALOG #	VENDOR
Fisherbrand™ Large-Orifice Pipet Tips, 1 to 200µL or equivalent	02-707-134	
Thermo Scientific™ Nalgene™ Rapid-Flow™ Sterile Disposable Bottle Top Filters with PES Membrane	09-741-07	Fisher Scientific
Drummond™ Fixed-Volume Microdispensers or equivalent	21176F	
Gilson™ PIPETMAN Classic™ Pipets- F123601 or equivalent	F123601G	
Fisherbrand™ Petri Dishes with Clear Lid or equivalent	FB0875713A	
Invitrogen™ EVOS™ XL Core Imaging System	12-562-751	Fisher Scientific
Bal Supply Cell Counter or equivalent	02-670-14	
Corning 4492 or equivalent	07-200-619	Fisher Scientific
Thermo Scientific™ Nunc™ Non-treated Flasks	12-566-85	Fisher Scientific

SAFETY WARNINGS

Please see attached SDS (Safety Data Sheet) for hazards and safety warnings.

Ciprofloxacin Hydrochloride Ciprofloxacin SDS-2014Jul22.pdf

Precautionary statements:

- P280 - Wear protective gloves and eye/face protection
- P305 + P351 + P338 - IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
- P337 + P313 - If eye irritation persists: Get medical advice/attention.
- P273 - Avoid release to the environment.

GemCell™ U.S. Origin Human Serum AB GemCell Human Serum AB.pdf

GemCell™ human serum AB is collected from healthy male donors of the AB serotype at FDA-licensed facilities in the United States.
Hazardous Components:

- Biohazard contains human source material. Handle as though capable of transmitting infectious agents.
- Toxicity: Not Established.

Target Organs/Systems: Product could possibly irritate the skin, eyes and respiratory system. Do not ingest this product.

Dimethyl sulfoxide (DMSO) DMSO_MSDSAction.pdf

- Hazard statement(s): Combustible liquid.
- Precautionary statement(s): Keep away from heat/sparks/open flames/hot surfaces. No smoking. Wear protective gloves/ protective clothing/ eye protection/ face

Citation: Integrated Islet Distribution Program (06/18/2020). Islet Culture and Preparation for Cold Shipping. https://dx.doi.org/10.17504/protocols.io.bfsfjnbn

protection.

- DMSO itself is not toxic but it can be a carrier of chemicals, viruses, etc. into the skin

Dithizone (DTZ) Dithizone_MSDSAction.pdf

- Hazard Statement(s): Causes skin irritation. Causes serious eye irritation.

Precautionary statement(s): Wash skin thoroughly after handling. Wear protective gloves/ eye protection/ face protection.

BEFORE STARTING

References:

- Prodo Labs, Inc. Protocols and Website: http://www.prodolabs.com
- PIM(R): https://prodolabs.com/pimr/ PIMRTechNotes.pdf

Scharp DW, Arulmoli J, Morgan K, Sunshine H, Hao E: Advances in Human Islet Processing: Manufacturing Steps to Achieve Predictable Islet Outcomes from Research Pancreases. OBM Transplantation 2019 Feb, Volume 3, Issue 1(Current Advancement of Islet Cell Transplantation in the Treatment of Diabetes Mellitus).

Ricordi C, Gray DW, Hering BJ, Kaufman DB, Warnock GL, Kneteman NM, Lake SP, London NJ, Socci C, Alejandro R, et al.: Islet isolation assessment in man and large animals. Acta Diabetol Lat 1990 Jul-Sep, 27(3):185-195.

Preparation of Supplies and Reagents

1

Laboratory Supplies:

The following supplies are necessary for the preparation of flasks for human islet culture prior to distribution.

- Islet preparation and distribution
- Wide mouth pipettes and pipettor
- Culture flasks- T-175 non-coated flasks
- Sampling wide-bore pipette tip and pipettor (Gilson or Drummond)
- 37°C CO_2 Incubator
- Conical tubes, 250 ml, sterile
- Routine lab supplies for transferring, media changing and counting islets.

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

- Human AB Serum (ABS) HI
- PIM(G)®
- PIM(R)®
- Ciprofloxacin Hydrochloride

3 **Receipt of Supplies:**

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

- The Prodo Labs PIM(R) should be stored, in the dark, between **2 °C** and **8 °C** upon receipt but is stable at room temperature.

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- The Gemini AB serum and the PIM(G) vials should be stored at **-5 °C** to **-20 °C** in the dark.
- The Ciprofloxacin should be stored as indicated by manufacturer (Corning at room temperature OR MP Biomedicals at 4°C). Aliquots of Ciprofloxin can be prepared prior to the isolation. Prepare the Ciprofloxacin according to the directions in the table. Filter sterilized suspension aliquots should be stored at **-5 °C** to **-20 °C** .

Pre-Preparation of Ciprofloxacin Powder for Addition to Media

- Remove 0.5 gm (500mg) of ciprofloxacin hydrochloride from the bottle and QS to 50mL with distilled water. This will give a stock concentration of 10mg/ml.
- Mix with a stir bar and stirring plate until totally dissolved.
- Filter sterilize the solution using a 0.2µM filter.
- Aliquot into sterile tubes, 5mL samples, label, and freeze for later use.
- The expiration date of the solution is indicated on the Certificate of Analysis and/or the bottle. Document expiration date as date of CoA.
- Diluted solution is good for 1 year frozen (if less than CoA expiration date) and 1 month thawed.

Record Ciprofloxacin preparation on ***Attachment 1: Solutions Preparation Sheet***, *of this SOP.*

Attachment 1-Solutions preparation Sheets.pdf

4 Preparation of PIM(R) Media:

- Prepare one 500 mL bottle of PIM(R) media prior to the isolation
- Thaw and add 5 mL of PIM(G)
- Thaw and add 25 mL of AB serum (5% v/v)
- Thaw and add 0.5 mL of prepared ciprofloxacin sterile aliquot
- Once all additives have been added to the bottle of PIM(R), it is now referred to as PIM(R) complete.
- Note: If culture for IIDP distribution is greater than 250,000 IEQ, a second bottle of PIM(R) complete will need to be prepared.

If PIM(R) complete is to be used from a previous isolation, it must have been filter sterilized at the end of the previous use. The media will expire within 30 days, once it has been fully supplemented.

Record media preparation on ***Attachment 1: Solutions Preparation Sheet***, *of this SOP.*

Post Purification Culture of Islet with ≥70% Purity

5

Post purification, all pooled islets with > 70% purity should be brought up in 200 mL of PIM(R) in a 250 mL conical.

- Thoroughly mix suspension by either pouring between 2 conicals or inverting one conical at least 3 times.

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- Quickly removing cap and count samples should be taken by a second technician.
- Take replicate 100-200 µl sample volumes from 100 mL final prep using a sampling wide-bore pipette tip and pipettor (Gilson or Drummond). Two duplicate counts should be performed by two separate technicians or 2 separate counts by one technician.
- Add 3 drops (30 µL) of the DTZ solution to the islets sample and allow staining for 1 – 2 minutes at room temperature. Cover the bottom of the counting dish with DPBS to approximately ½ the height of the dish. Count the islets under the microscope as described in the SOP: ***Qualitative & Quantitative Assessment of Human Islets for Distribution Using Dithizone (DTZ) V.2*** https://www.protocols.io/view/qualitative-quantitative-assessment-of-human-islet-bhdjj24n

Note: 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)

- 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.

Record counts on Attachment 2-Islet Tabulation Counting Sheet for Culturing Islets using Attachment 3- Islet Ranking Guide for Ranking, of this SOP.

Attachment 2-Islet Tabulation Counting Sheet for Culturing Islets.xlsx

Attachment 3-Islet Ranking Guide.pdf

6 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.

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

7 Calculate the amount of suspension needed to aliquot 20,000 IEQ per T-175 flask.

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

8 Calculate the number of flasks needed to culture the islets for broadcast at 20,000 IEQ per flask.

- Example: 250,000 IEQ/20,000 IEQ=12.5 flasks (or 200 mL/16 mL=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.
- Example: 12.5 → 13 Flasks; 200 mL/13 flasks = 15.4 mL islet suspension/flask.
- Determine the amount of media needed to prewet each flask. Example: 40 mL - 15.4 mL islet suspension = 24.6 mL fresh PIM(R).

9 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).

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

10 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.

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

12 Rinse the conical after the (marked) final flask has been loaded with 3 mL of extra media. Repeat. Total volume will be 40 mL

13 After dispensing all of the islets, place all the flasks in the incubator and set at 37°C ± 0.5°C and 5% ± 0.5% CO_2 and culture for at least 48 hours.

Note: Possible exceptions for culture time.

Attachment 4- Tips for Determining Islet Quality for Shipment.pdf

14 Between 12 and 18 hours after culture begins, perform a 50% media change on all flasks listed in Step 16-26 under ***Half Media Change Procedure.***

Post Purification Culture of Islet with <70% Purity

15 Follow all steps outlined in steps 5-14, however determine the concentration of the cultured islets and the number of flasks needed in Step 7 (bullet point) by multiplying the total flasks by the percent purity.

- Example: If part of the prep has a 50% purity and 50,000 IEQ; then 50,000 IEQ/(20,000IEQ * 50%) = 5 flasks.
- Determine all other values based on this calculation.

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

Half Media Change Procedure

16

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.)*

17 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. **00:00:00**

18 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.

19 Rock the flasks gently back and forth keeping them horizontal, to get any islets that are loosely attached to the bottom

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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).

20 Arrange a 50 mL conical rack supported by a 250 mL 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 250 mL and 50 mL conical racks for support, resting them on the longer edge of the 50 mL 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. **00:00:00**

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

22 Go to the middle point of the media in one flask and take a 1 mL 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.

23 While maintaining the position of the flask (as in step 19) aspirate 50% of the used media (20 mL) 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.

24 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.

25 Pipette 20 mL of fresh PIM(R) into each flask after aspiration and tighten caps.

26 Once media change is completed for all the flask(s), check aspirate for any greater than 50 µm islets and if any are found, transfer to a flask. Confirm all ventilated flask caps are tight and return to incubator set to 37°± 0.5°C.

27 *Using a 0.2 µm filter, filter sterilize any media remaining in the fresh PIM(R) complete bottle. The media will expire within 30 days once it has been fully supplemented.*

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