Version 2

Jun 11, 2020

Sentinel Flask Preparation for Cold Shipping Protocol of Human Islets V.2

Integrated Islet Distribution Program[1]

[1]Integrated Islet Distribution Program, City of Hope

1 Works for me dx.doi.org/10.17504/protocols.io.bhdpj25n

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

ABSTRACT

To establish a standardized method for the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) sponsored research in the Integrated Islet Distribution Program (IIDP) for preparing a sentinel flask of human islets in order to monitor sterility and islet integrity at the production center. This is a representative sample of the islet preparation that has been distributed to investigators and is held at the distributing center as a quality control sample. The islets are cultured in the same shipping media used for islet shipment, for 18 hours at 4-8°C, simulating the cold shipping conditions followed by 2 days at 37°C culture. The flask of islets are verification in the event of a dispute over sterility or islet quality.

Integrated Islet Distribution Program (IIDP) (RRID:SCR_014387)

EXTERNAL LINK

https://dx.doi.org/10.17504/protocols.io.bac5iay6; https://iidp.coh.org/Investigators/Policies-Standard-Operating-Procedures

ATTACHMENTS

Prodo Media-Preparation-and-Use-Instructions-1.pdf | GemCell Human Serum AB.pdf | Attachment 1-Solutions preparation Sheets.pdf | Attachment 2-Sentinel Flask Documentation Sheet.pdf

DOI

dx.doi.org/10.17504/protocols.io.bhdpj25n

PROTOCOL CITATION

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KEYWORDS

Human Islets, culture, sentinel flask, IIDP, QA, Quality Assurance, Islet Equivalent, Prodo Labs

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GUIDELINES

- ***Integrated Islet Distribution Program (IIDP) (RRID:SCR_014387):*** 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 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., IIDP Principal Investigator leads CC staff to coordinate the activities of the IIDPand assists the participating centers and investigators in the distribution of human islets.

- ***Islet Equivalent (IEQ):*** An IEQ is defined as an islet with a diameter of 150 microns. The number of IEQ in each size class is calculated by multiplying the number of islets by a conversion factor for each micron size.

- ***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.

- ***Sentinel Flask:*** A representative sample of the islet preparation that has been distributed to investigators that is held at the distributing center as a quality assurance sample. This sample should be held in the 30 mL shipping flask at the center and should contain at least 100 IEQ cultured in the same shipping media used for islet shipment. *One flask must be prepared for each purity batch of islets distributed.*

MATERIALS

NAME	CATALOG #	VENDOR
Human AB Serum (ABS) HI	100-512; Heat Inactivated	Gemini Bioproducts
PIM(G)® (5 mL Glutamine/Glutathione)	PIM(G)®	Prodo Laboratories, Inc
PIM(T)®	PIM(T)®	Prodo Laboratories, Inc
Corning™ Ciprofloxacin Hydrochloride	MT61277RG (Corning™ 61277RG)	Fisher Scientific

MATERIALS TEXT

Invitrogen™ EVOS™ XL Core Imaging System or equivalent
Inverted Microscope

Light Microscope AMEX1000

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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

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

Drummond™ Fixed-Volume Microdispensers or equivalent
Drummond 3000385

Drummond™ 21176F
Volumetric Range 100/200UL with borosilicate glass bores

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Bottle Top Filters with PES Membrane or equivalent
0.2 µm Bottle Top Filter
Thermo Scientific™ Nalgene™ Rapid-Flow™ Sterile Di 09-741-09
0.2 µm membranes for sterile filtration; PES is the best membrane for cell culture fluids; lowest protein binding to maintain protein balance, lowest extractables to maintain media purity

Thermo Scientific™ Nalgene™ Square PETG Media Bottles with Closure
30 mL PETG Media Bottles with Closure
Thermo Scientific™ Nalgene™ 03-311-1V
30 mL PETG Media Bottles

SAFETY WARNINGS

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

Ciprofloxacin Hydrochloride
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 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.

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- Toxicity: Not Established.

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

BEFORE STARTING

Prodo Labs, Inc. Protocols and Website: http://www.prodolabs.com

Ricordi, C. (1992). Pancreatic Islet Cell Transplantation.. Austin: R.G. Landes Company, 1992:137-138..

Ricordi, C. (Ed). (1995). Methods in Cell Transplantation.. Austin: R.G. Landes Company, 1995: Section G..

SUPPLEMENTAL MATERIALS

1 The following supplies are necessary for the preparation of Sentinel Test Flasks for human islet distribution.

- Islet preparation for distribution
- Wide mouth pipettes and pipettor
- 30 mL shipping bottles - 1 per islet purity batch broadcasted.
- Shipping media that is to be used for distribution as decribed below.

MEDIA PREPARATION

2 Shipping media that is to be used for distribution is prepared as described below.

- The Prodo Labs PIM(T) should be stored between 2° and 8°C upon receipt but is stable at room temperature.
- The (heat inactivated) Gemini AB serum and the PIM(G) vials should be stored at -5° to -20°C.

3 The Ciprofloxacin powder can be stored on the shelf but filter sterilized suspension aliquots should be stored at -5° 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.

4 Prepare 1 bottle of PIM(T) media prior to the collection by adding the following:

- Thaw and add 5 mL of PIM(G).
- Add 12.5 mL of AB serum (2.5%v/v).
- Add 0.5 mL of prepared sterile ciprofloxacin aliquot.
- Once all additives have been added to the bottle of PIM(T), it is now referred to as PIM(T) complete.

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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. The media will expire within 30 days, once it has been fully supplemented.

SENTINEL FLASK PREPARATION

5 Assemble all items described in the *Material and Media Preparation Sections*.

6 At the time of preparation for islet distribution, the cultured islets are consolidated and counted. Calculate the volume of suspension that is needed from the islet preparation to provide at least 100 IEQs.

The formula will be:

$TotalIEQ/(TotalmL * 1000\mu L) = xIEQ/\mu L$ then

$100IEQ/xIEQ/\mu L = y\mu L$

7 Thoroughly resuspend the preparation with a large mouth pipet and remove the calculated aliquot. Place in the 30mL shipping flask. Top with appropriate amount of media using the same batch of PIM(T) complete that will be used for distribution. *Repeat for each purity batch that is being distributed.*

8 Store this/these Sentinel QA Flask(s) between 4°C and 8°C for approximately 18 hours after which the flask(s) should be moved to a 37°C incubator for an additional 2 days.

Note: This culture procedure should simulate the average shipping time followed by culture by an investigator. A lab refrigerator should be appropriate for the first 18 hours of cold temperature culture.

9

Observe under a microscope daily for visual islet integrity, quality, and sterility. Record results on *Attachment 2 - Sentinel Flask Documentation Sheet.*

10 Alert the IIDP and the recipients of the distributed islets if there is a problem with the sterility of the preparation. In which case, send a copy of the *Attachment 1-Sentinel Flask Documentation Sheet to IIDP.*

The sentinel flask is mainly for the documentation of the preparation by the distribution center in circumstances where islet sterility, quality or integrity of the distributed islets is questioned by a recipient. Photographs may be required to document the visual state of the sentinel flask if there is a challenge.

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