Jan 12, 2021

Islet Embedding for Histology

IIDP-HIPP [1]

[1]Integrated Islet Distribution Program and Human Islet Phenotyping Program

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

Integrated Islet Distribution Program and Human Islet Phenotyping Program
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IIDP-HIPP

ABSTRACT

This Standard Operating Procedure (SOP) is based on the Vanderbilt Human Islet Phenotyping Program (HIPP) Immobilization of Islets in 3-D Gel for Histology. This SOP provides HIPP procedure for embedding islets in Collagen I gel for cryosectioning and histological staining.

This SOP defines the assay method used by the Human Islet Phenotyping Program (HIPP) for quantitative and qualitative determination of the Purified Human Pancreatic Islet product, post-shipment, manufactured for use in the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK)-sponsored research in the Integrated Islet Distribution Program (IIDP).

This Standard Operating Procedure (SOP) #: HIPP-06-v02

DOI

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KEYWORDS

HIPP, Islet Embedding, cryosection, histological stain

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Dec 17, 2020

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PROTOCOL INTEGER ID

45678

GUIDELINES

- ***Integrated Islet Distribution Program (IIDP) (RRID:SCR_014387):*** The IIDP is a grant funded 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 Scientific Panel 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. and Carmella Evans-Molina, M.D., Ph.D. serve as Co-Principal Investigators (Co-PIs) for the IIDP Program located within the Department of Diabetes and Cancer Discovery Science at COH to coordinate the activities of the IIDP and Human Islet Phenotyping Program (HIPP). Dr. Niland, contact PI, oversees the daily activity of the IIDP staff, provides informatics/ biostatistical input, and subcontracts with the Islet Isolation Centers (IICs) to ensure the delivery of the highest quality human islets to IIDP approved investigators. Dr. Evans-Molina serves as the liaison to the HIPP, interacting closely to ensure that extensive, high quality phenotypic data are collected on islets distributed by the IICs. She also facilitates the delivery of this information to both the IICs and the IIDP-approved investigators, while responding to questions, issues, or suggestions for further HIPP enhancements.

- ***Human Islet Phenotyping Program (HIPP):*** The HIPP is a subcontracted entity of the IIDP through the COH and Vanderbilt University. The HIPP is directed by Marcela Brissova, Ph.D. and is responsible for performing specific standardized quality control assays agreed upon by both the IIDP and the HIPP, in order to provide enhanced, quality data on the human islets post-shipment, to the IIDP. The results of these assays will be approved by the CC and posted on the IIDP website for both the centers and the approved investigators.

References:

Dai C, Brissova M, Hang Y, Thompson C, Poffenberger G, Shostak A, et al. Islet-enriched gene expression and glucose-induced insulin secretion in human and mouse islets. Diabetologia. 2012 Mar;55(3):707–18. PMCID: PMC3268985. https://pubmed.ncbi.nlm.nih.gov/22167125/

Guo S, Dai, Guo M, Taylor B, Harmon JS, Sander M, et al. Inactivation of specific β cell transcription factors in type 2 diabetes. J Clin Invest. 2013 Aug;123(8):3305–16. PMCID: PMC3726150. https://pubmed.ncbi.nlm.nih.gov/23863625/

MATERIALS TEXT

1. PBS (phosphate buffered saline) with no Ca/Mg, 1X (Invitrogen 14190-144)
2. Collagen I stock (BD 354249)
3. DMEM, 5X (Dulbecco modified Eagle's minimal essential medium, Gibco 31600-034)
4. HEPES (N-2-hydroxyethylpiperazine-N-2-ethane sulfonic acid, Sigma H0887)
5. $NaHCO_3$ (sodium bicarbonate, Sigma S8761)
6. Sucrose (Fisher Scientific BP220-1)
7. 16% Paraformaldehyde (Electron Microscopy Science 15710)
8. **1.5 mL** Centrifuge Tube (Fisher Scientific 05-408-129)
9. P-200 Pipet Tips, Sterile (Fisher Scientific P-2069)
10. Wide-bore pipet tips for transferring islets (Fisher Scientific 212362C)
11. 96-well Plate (Falcon 353072)

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12. Needle, 28G, insulin syringe (Exel International 26027)
13. 12-well plate (Fisher Scientific 351143)
14. Cryomolds (Tissue-Tek 4557)
15. OCT (Optimum Cutting Temperature compound, Tissue-Tek 4583)
16. Plus Gold Slides (Fisher Scientific 15-188-48)
17. Cryostat (Leica)
18. Adjustable tilt rocker (LabNet)
19. Stereomicroscope (Olympus)
20. Digital Camera
21. **-80 °C** Freezer (Thermo Scientific)

PBS (phosphate buffered saline) with no Ca/Mg **Thermo Fisher Scientific Catalog #Invitrogen 14190-144**

Collagen I stock **Corning Catalog #BD 354249**

DMEM 5X **Thermo Fisher Scientific Catalog #Gibco 31600-034**

HEPES **Sigma Aldrich Catalog #Sigma H0887**

NaHCO3 **Sigma Aldrich Catalog #Sigma S8761**

Sucrose **Fisher Scientific Catalog #BP220-1**

16% Paraformaldehyde **Electron Microscopy Sciences Catalog #15710**

Centrifuge Tube
1.5mL
Fisher Scientific **05-408-129**

Pipet Tips
P-200
Sterile **Fisher Scientific P-2069**

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Wide-bore pipet tips

Fisher Scientific **212362C**

PDF

96-well Plate

Falcon **353072**

Needle, insulin syringe
28G

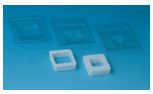
Exel International **26027**

12-well plate

Falcon **351143**

Cryomolds

Tissue-Tek **4557**



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OCT (Optimum Cutting Temperature compound)

Tissue-Tek **4583**

Plus Gold Slides

Fisher Scientific **15-188-48**

Cryostat

Leica **Cryostat**

Adjustable tilt rocker

LabNet **35 Deluxe Rocking Platform**

Stereomicroscope

Olympus **Stereomicroscope**

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Freezer
-80°C

Thermo Scientific | ultra-low freezers (ULT)

SAFETY WARNINGS

Sucrose ***(Fisher Scientific BP220-1)***

May cause skin, eye, and respiratory tract irritation. Wear personal protective equipment. Ensure adequate ventilation. Avoid contact with skin, eyes and clothing. Avoid ingestion and inhalation. Use personal protective equipment. Wash off immediately with plenty of water for at least 15 minutes. Get medical attention immediately if symptoms occur. Rinse immediately with plenty of water, also under the eyelids, for at least 15 minutes. Obtain medical attention. Move to fresh air. If breathing is difficult, give oxygen. If breathing is difficult, give oxygen. Get medical attention immediately if symptoms occur.

16% Paraformaldehyde ***(Electron Microscopy Science 15710)***

16% Paraformaldehyde 15710.pdf

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This Standard Operating Procedure (SOP) #: HIPP-06-v02

Procedures

1 Islet Immobilization and Fixation

Citation: IIDP-HIPP (01/12/2021). Islet Embedding for Histology. https://dx.doi.org/10.17504/protocols.io.bqunmwve

1.1 Prepare Collagen I working solution (**1 mL**) by combining **375 µl** Collagen I stock, **355 µl** sterile water, **20 µl** HEPES, **50 µl** $NaHCO_3$, **200 µl** 5X DMEM.

1.2 Using a P-1000 pipette and wide-bore tips, transfer an aliquot of islet suspension containing approximately 500 IEQs into a **1.5 mL** centrifuge tube and centrifuge at 1000 rpm for 1 min. Aspirate the supernatant. If islet medium is rich in serum, wash the islets once with 1X PBS.

1.3 Add **150 µl** of Collagen I working solution to the islet pellet and transfer the mixture into a 96-well plate. Place the plate in a tissue culture incubator set to **37 °C** . Incubate for 90 min and Collagen I will form into a gel. While gel is solidifying, islets will settle by gravity at the bottom of the gel and form a monolayer.

1.4 Place 96-well plate on ice and add ice-cold 4% paraformaldehyde/1X PBS on top of the gel to fill up the well. Fix for 10 min on ice.

1.5 Using a 28 G needle (**0.3 mL** insulin syringe) loosen up the gel containing islets from the sides of the well. Then, using a fine spatula transfer the gel into a 12-well plate containing **3 mL** of ice-cold 4% paraformaldehyde/1X PBS per well. ***Be very careful while you do this because the gel is relatively soft.*** Fix the islets on ice for an additional 15 min under very mild agitation using an adjustable tilt rocker (low setting).

1.6 Gently aspirate the fixative and wash the gel with **3 mL** of 1X PBS on ice for 20 min under very mild agitation using an adjustable tilt rocker (low setting).

1.7 Repeat step 1.6 two times.

2 Islet Embedding and Sectioning

2.1 After the last wash, add **3 mL** of 30% sucrose/1X PBS. Allow the gel to equilibrate for 2-3 hours or overnight at **4 °C** .

2.2 Lift the gel from the dish using a fine spatula and place it into a standard cryomold that is half-way filled with OCT compound. While doing this, orient the gel such that **the bottom of the gel (where the islets are) is facing the bottom of the cryomold**. Add more OTC to fill up the cryomold. Take an image of the gel on an Olympus SZX12 microscope at 16x. Example of islets embedded in Collagen I is shown in ***Figure 1***.

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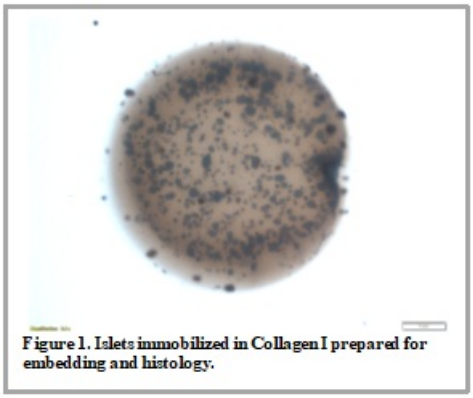

Figure 1. Islets immobilized in Collagen I prepared for embedding and histology.

2.3 Place the embedded gel in a **-80 °C** freezer. When OCT compound is frozen, wrap the block in pre-labeled aluminum foil, place it a zip-lock bag, seal and store at **-80 °C** .

2.4 Cut 8-µm cryosections and plate two sections per slide on *Plus Gold* slides. Transfer slides and blocks on dry ice back to the HIPP and store in a **-80 °C** freezer.

2.5 Enter slides and blocks into the Vanderbilt HIPP electronic inventory system and upload images of islet gels to the IIDP-HIPP database.

Deviations and Resolution

3 Document any deviations that occurred during this protocol that affect the final results and report with the analysis of the assay.

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