Feb 10, 2021

Assessment of Human Islet Composition and Acinar Cell Component by Immunofluorescence Staining

IIDP-HIPP [1]

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

1 Works for me dx.doi.org/10.17504/protocols.io.brdfm23n

Integrated Islet Distribution Program and Human Islet Phenotyping Program
Tech. support email: heather.durai@vumc.org

IIDP-HIPP

SUBMIT TO PLOS ONE

ABSTRACT

This Standard Operating Procedure (SOP) is based on the Vanderbilt Human Islet Phenotyping Program Core Facility (VHIPP) Immunofluorescence Staining Procedure. This SOP provides HIPP procedure for immunofluorescent staining, imaging, and analysis of islet preparations.

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-07-v02

DOI

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PROTOCOL CITATION

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KEYWORDS

Human Islet Composition, Acinar Cell Component, Immunofluorescence Staining, HIPP, IIDP

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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 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. 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 phenotyping 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.

- ***Cryosections:*** Sections of a tissue/cells embedded in optimal cutting temperature (OCT) compound and frozen **-80 °C** .

- ***Indirect Immunofluorescence Staining:*** Immunohistochemical procedure based on antigen detection by flourescence in histological sections using a combination of primary and secondary antibodies where the primary antibody is directed to the antigen of interest and the fluorescently-conjugated secondary recognizes species where the primary antibody was raised. Histological sections are viewed using a microscope system equipped with an appropriate light source and filter set to allow for visualization of fluorescence tissue staining.

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 C, 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

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1. PBS (phosphate buffered saline) with no Ca/Mg, 1X (Invitrogen 14190-144)
2. BSA (bovine serum albumin, Sigma A-6003)
3. SlowFade Gold (Molecular Probes S36938)
4. Triton X-100 (BioRad 1610407)
5. Normal Donkey Serum (NDS, Jackson Immuno Research 017-000-121)
6. 4',6-diamidino-2-phenylindole (DAPI, ThermoFisher Scientific D1306)
7. Kartell Staining Chambers (VWR 25460-907)
8. PAP Marker (Research Products International 195506)
9. **4 °C** Refrigerator (ArcticTemp)

PBS (phosphate buffered saline) with no Ca/Mg 1X **Thermo Fisher Scientific Catalog #Invitrogen 14190-144**

BSA (bovine serum albumin) **Sigma Aldrich Catalog #A-6003**

SlowFade Gold (Molecular Probes) **Thermo Fisher Scientific Catalog #S36938**

Triton X-100 **Bio-rad Laboratories Catalog #1610407**

Normal Donkey Serum **Jackson Immunoresearch Catalog #017-000-121**

4, 6-diamidino-2-phenylindole (DAPI) **Thermo Fisher Scientific Catalog #D1306**

Kartell Staining Chambers

VWR 25460-907

Marker

PAP 195506

4°C Refrigerator

ArcticTemp None

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SAFETY WARNINGS

Triton X-100 (*BioRad 1610407*)

Triton X-100.pdf

Preparation of Reagents

1 Preparation of Reagents for Immunofluorescence Staining of Islet Cryosections

1.1 ***10% Triton X-100 stock (*** **30 mL** ***)*** – combine **3 mL** Triton-X-100 and **27 mL** 1X PBS. Mix on shaker for 30 min or until Triton X-100 is completely dissolved and store at **4 °C** for up to 1 month.

1.2 ***Permeabilization Solution (0.2% Triton,*** **50 mL** ***)*** – combine **1 mL** of 10% Triton stock and **49 mL** 1X PBS.

1.3 ***Blocking Buffer (5% NDS,*** **4 mL** ***)*** – combine **0.2 mL** NDS and **3.8 mL** 1X PBS.

1.4 ***Antibody Buffer (*** **10 mL** ***)*** – combine **0.1 g** BSA, **0.1 mL** 10% Triton stock, and **9.8 mL** 1X PBS.

1.5 ***DAPI staining solution (1:25,000,*** **50 mL** ***)*** – combine **2 µl** DAPI stock (5mg/mL) and **50 mL** 1X PBS.

Procedure

2 Immunofluorescence Staining Procedure on Islet Cryosections

2.1 Use freshly-made antibody incubation buffers, and wash buffers. Steps 2.3, 2.4, 2.8, 2.10, 2.11 can be done in Kartell Staining Chambers.

2.2 Let the frozen sections thaw at room temperature and air-dry for about 30 minutes.

2.3 Wash the sections with **50 mL** 1X PBS 3 times for 5 minutes to remove the OCT.

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2.4 Permeablize the tissue section with 0.2 % Triton for 15 minutes at room temperature.

2.5 Wash the tissue in **50 mL** 1X PBS 3 times for 3-5 minutes.

2.6 Draw circles or rectangles around the sections with PAP marker and let them dry for about 5 minutes.

2.7 Block the sections with 5% normal donkey serum (made from 100% stock)/1X PBS at room temperature for 90 minutes in a humidified chamber.

2.8 Aspirate the blocking solution, add primary antibodies (**Table 1**) diluted in 0.1% Triton-X-100 (made from 10% Triton stock)/1% BSA/1X PBS and incubate in a humidified chamber overnight at **4 °C** .

Table 1. List of primary and secondary antibodies for assessment of islet cell composition and endocrine/acinar cell composition

Primary Antibody	Vendor	Product number	Final Dilution	Secondary Antibody	Vendor	Product number	Final Dilution
C-peptide (rat)	Developmental Studies Hybridoma Bank	GN-ID4 RRID:AB_2631151	1:100	Rat IgG-Cy2 (donkey)	Jackson ImmunoResearch	712-225-150 RRID:AB_2340673	1:500
				Rat IgG-Cy5 (donkey)	Jackson ImmunoResearch	712-175-150 RRID:AB_2340671	1:200
Glucagon (mouse)	Abcam	ab10988 RRID:AB_297642	1:250	Mouse IgG-Cy3 (donkey)	Jackson ImmunoResearch	715-165-150 RRID:AB_2340813	1:500
Glucagon (rabbit)	Cell Signaling Technology	2760S RRID:AB_659831	1:100	Rabbit IgG-Cy5 (donkey)	Jackson ImmunoResearch	711-175-152 RRID:AB_2340607	1:200
Somatostatin (goat)	Santa Cruz Biotechnology	sc-7819 RRID:AB_2302603	1:500	Goat IgG-Cy5 (donkey)	Jackson ImmunoResearch	705-175-147 RRID:AB_2340415	1:200
HPX1 (mouse)	Novus Biologicals	NBP1-18951 RRID:AB_1625456	1:100	Mouse IgG-Cy3 (donkey)	Jackson ImmunoResearch	715-165-150 RRID:AB_2340813	1:500

Antibody Information

Primary Antibodies:

C-peptide (rat): GN-ID4 RRID:AB_2631151
Glucagon (mouse): ab10988 RRID:AB_297642
Glucagon (rabbit): 2760S RRID:AB_659831
Somatostatin (goat): sc-7819 RRID:AB_2302603
HPX1 (mouse): NBP1-18951, RRID:AB_1625456

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Secondary Antibodies:

Rat IgG-Cy2 (donkey): 712-225-150 RRID:AB_2340673
Rat IgG-Cy5 (donkey): 712-175-150 RRID:AB_2340671
Mouse IgG-Cy3 (donkey): 715-165-150 RRID:AB_2340813
Rabbit IgG-Cy5 (donkey): 711-175-152 RRID:AB_2340607
Goat IgG-Cy5 (donkey): 705-175-147 RRID:AB_2340415
Mouse IgG-Cy3 (donkey): 715-165-150 RRID:AB_2340813

2.9 Aspirate the primary antibodies and wash the sections with 1X PBS three times for 10 minutes each.

2.10 Add secondary antibodies (**Table 1**) diluted in 0.1% Triton/1% BSA/1X PBS and incubate for 1.5 hours at room temperature in a humidified chamber.

2.11 Aspirate the secondary antibody and counterstain slides with 1:25,000 DAPI/PBS for 10 minutes at room temperature.

2.12 Remove from DAPI and wash the sections with 1X PBS three times for 15 minutes each.

2.13 Mount the sections with SlowFade Gold mounting medium.

Imaging and Analysis

3 Imaging and Analysis of Fluorescently Labeled Islet Cryosections

3.1 Capture images of islet sections using a high-resolution whole slide scanning system (ScanScope FL, Aperio/Leica) connected to a web-based digital slide repository powered by eSlide Manager and housed in the Vanderbilt University Medical Center data center (*examples of islet images are shown in **Figures 1** and **2***).

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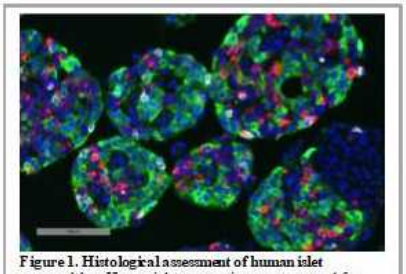

Figure 1. Histological assessment of human islet composition. Human islet preparation was processed for cryosections. (**A**) Each specimen label is captured by slide scanner and contains information about UNOS ID, disease type, donor age, gender, and islet isolation center. (**B - D**). Islet composition was determined by immunofluorescence for islet markers (β cells – C-peptide, green; α cells – glucagon, red; δ cells – somatostatin; white, DAPI – blue) using a tissue classifier algorithm (Halo™).

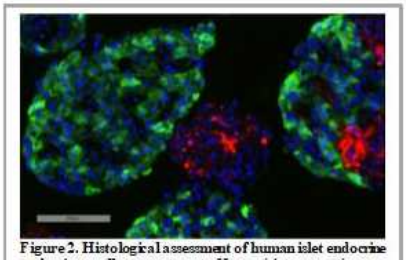

Figure 2. Histological assessment of human islet endocrine and acinar cell compartments. Human islet preparation was processed for cryosections. (**A**) Each specimen label is captured by slide scanner and contains information about UNOS ID, disease type, donor age, gender, and islet isolation center. (**B - D**) Sections were labeled for islet markers (C-peptide, glucagon, and somatostatin – green), acinar cell marker Hpx1 (red), counterstained with DAPI (blue) and then analyzed with algorithm (Halo™) to measure endocrine cell compartment.

3.2 Using a tissue classifier algorithm (Halo™, Indica Labs) analyze islet images (50 -100 islets/labeling experiment) to provide a quantitative assessment of the islet cell composition and endocrine/acinar cell compartments for a given human islet preparation (*examples of quantitative islet assessment are shown in the legend of* ***Figures 1*** *and* ***2***).

Data Storage and Reporting

4 Data Storage and Reporting

4.1 To facilitate data management and ensure data security, the Vanderbilt HIPP uses an institutional server-based platform for data storage and analysis.

4.2 Upon analysis completion (within 14 business days) annotated images containing metadata and image analysis outputs will be uploaded to the IIDP-HIPP database and immediately disseminated to IIDP-affiliated investigators and islet isolation centers.

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