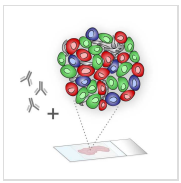

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Dec 02, 2021

Immunofluorescent Staining of Mouse Pancreas for Islet Cell Mass Analysis V.1

Islet and Pancreas Analysis Core[1]

[1]Vanderbilt Diabetes Research Center

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dx.doi.org/10.17504/protocols.io.bzvwp67e

Vanderbilt Diabetes Research Center

Islet and Pancreas Analysis Core
Vanderbilt Diabetes Research Center

This SOP defines the assay method used by the Vanderbilt Diabetes Center Islet and Pancreas Analysis (IPA) Core for quantitative determination of the islet cell composition and islet cell mass of mouse pancreas by immunofluorescent staining.

DOI

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https://dx.doi.org/10.17504/protocols.io.bzvwp67e

protocol ,

Nov 08, 2021

Dec 02, 2021

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In steps of

Mouse Pancreas Dissection and Fixation for Cryosectioning

General reagents:

1X Phosphate buffered saline without Ca/Mg **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, Dihydrochloride (DAPI) **Thermo Fisher Scientific Catalog #D1306**

Super HT PAP Pen **Research Products International (rpi) Catalog #195506**

10 mL BD Luer-Lok Syringe **BD Biosciences Catalog #302995**

Millex-GP Syringe Filter Unit, 0.22 µm **Emd Millipore Catalog #SLGP033RS**

Antibodies:

Insulin antibody (guinea pig polyclonal) **Fitzgerald Catalog #20-IP30**

Glucagon antibody (rabbit polyclonal) **Cell Signaling Technology Catalog #2760S**

Somatostatin antibody (goat polyclonal) **Santa Cruz Biotechnology Catalog #sc-7819**

Cy2 AffiniPure Donkey Anti-Guinea Pig IgG (H L) **Jackson Immunoresearch Catalog #706-225-148**

Cy3 AffiniPure Donkey Anti-Rabbit IgG (H L) **Jackson Immunoresearch Catalog #711-165-152**

Cy5 AffiniPure Donkey Anti-Goat IgG (H L) **Jackson Immunoresearch Catalog #705-175-147**

Equipment:

Staining chambers
Kartell **25460-907**

Data acquisition and analysis:

Aperio slide scanner
Leica **Scanscope FL**

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HALO® Image Analysis Platform
by Indica Labs

Reagent preparation

1 **10% Triton X-100 stock** (30 mL) – Combine 3 mL Triton-X-100 and 27 mL 1X PBS. Mix on shaker for **00:30:00** or until Triton X-100 is completely dissolved. Store at **4 °C** for up to 1 month.

2 **Permeabilization solution** (0.2% Triton, 50 mL) – Combine 1 mL 10% Triton stock and 49 mL 1X PBS.

3 **Blocking buffer** (5% Normal Donkey Serum, 4 mL) – Combine 0.2 mL NDS and 3.8 mL 1X PBS.

4 **Antibody buffer** (10 mL) – Combine 0.1 g BSA, 0.1 mL 10% Triton stock, and 9.8 mL 1X PBS. Filter solution through a 0.22 µm syringe filter and store at **4 °C** .

5 **DAPI staining solution** (1:25,000, 50 mL) – Combine 2 µl DAPI stock (5mg/mL) and 50 mL 1X PBS. Keep protected from light or prepare right before step 17.

6 **Wash buffer** (150 mL) - Combine 148.5 mL 1X PBS and 1.5 mL 10% Triton X-100 stock.

Immunofluorescent staining

7 Gather reagents for immunostaining, noting that steps 9-11, 15, and 17-18 can be performed in Kartell staining chambers (each holds ~50 mL).

> Always use freshly-made antibody and wash buffers (steps 4, 6); other reagents can be prepared ahead of time and stored at **4 °C** .

8 Let the frozen cryosections thaw at room temperature and air-dry for about **00:30:00** .

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9 To remove OCT, wash slides 3 times in 1X PBS for **00:05:00** , decanting or switching to a new chamber after each wash.

10 Permeabilize the tissue section with **permeabilization solution** for **00:15:00** at room temperature.

11 Wash the sections 3 times in 1X PBS for **00:05:00** , decanting or switching to a new chamber after each wash.

12 Draw rectangles around the sections with PAP pen and let them dry for about **00:05:00** .

13 Block the sections with **blocking buffer** at room temperature for **01:30:00** in a humidified chamber. Use a sufficient volume (approximately 200-300 µl) to ensure each section is entirely covered with buffer.

14 Aspirate the blocking buffer, add primary antibodies diluted in **antibody buffer** (see **Table 1**), and incubate in a humidified chamber **Overnight** at **4 °C** . Use a sufficient volume (approximately 200-300 µl) to ensure each section is entirely covered with antibody solution.

Table 1: Primary and secondary antibodies

Primary Antibody	Vendor	Product number	Final Dilution	Secondary Antibody	Vendor	Product number	Final Dilution
Insulin (guinea pig)	Fitzgerald Industries	20-IP30 RRID:AB_231770	1:250	Guinea Pig IgG-Cy2 (donkey)	Jackson ImmunoResearch	706-225-148 RRID:AB_2340467	1:500
Glucagon (rabbit)	Cell Signaling Technology	2760S RRID:AB_10698611	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

Table 1: Detailed information for primary and secondary antibodies.

15 Aspirate the primary antibody solution and wash the sections 3 times in **wash buffer** for **00:10:00** , decanting or switching to a new chamber after each wash.

16 Add secondary antibodies diluted in **antibody buffer** (see **Table 1**) and incubate for **01:30:00** at room temperature in a humidified chamber. Use a sufficient volume (approximately 200-300 µl) to ensure each section is entirely covered with antibody solution.

17 Aspirate the secondary antibody solution and incubate slides in **DAPI solution** to counterstain for **00:10:00** at room temperature.

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18 Wash slides 3 times in 1x PBS for **00:15:00** , decanting or switching to a new chamber after each wash.

19 Add a sufficient volume of SlowFade Gold mounting medium (approximately 5-10 µl) to each tissue section, ensuring tissue section will be entirely covered after medium spreads. Carefully mount coverslips, making sure to avoid creating air bubbles between the tissue and coverslip.

20 Blot away any excess mounting medium from slide edges and seal the coverslipped slides with nail polish on all edges. Allow slides to dry completely before imaging.

Imaging and analysis

21 Acquire images of stained pancreas sections using a high-resolution whole slide scanning system (Aperio ScanScope FL, Leica Biosystems). An example field of view is shown below in **Figure 1**.

22 Apply a tissue classifier algorithm (HALO® image analysis platform, Indica Labs) to quantify cross-sectional area of islet alpha (glucagon+), beta (insulin+), and delta (somatostatin+) cells. An example markup image is shown in **Figure 2**.

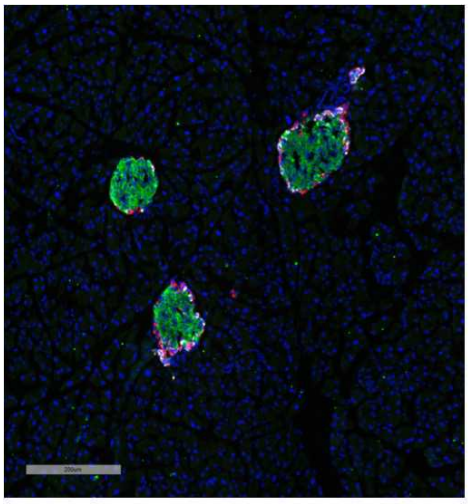

Figure 1. Fluorescent imaging of mouse pancreatic tissue. Tissue sections were labeled with islet markers (β cells- insulin, green; α cells- glucagon, red; δ cells- somatostatin, white; DAPI- blue.) High-resolution, whole-slide imaging of tissue was performed using the ScanScope FL scanning system.

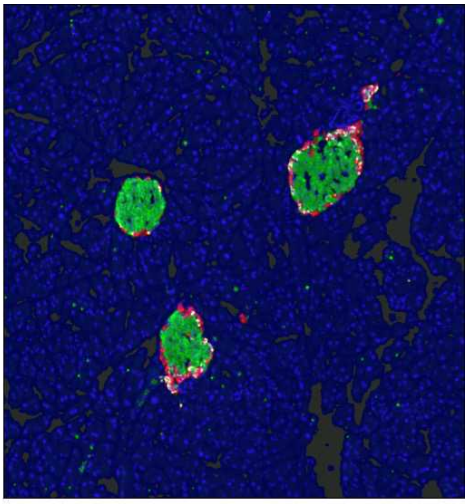

Figure 2. Analysis of mouse pancreatic islets using a tissue classifier algorithm. Islet composition and α, β, and δ cell mass were determined by using Halo™ software (Indica Labs.)

Figure 1 (above left). Fluorescent imaging of mouse pancreatic tissue.
Figure 2 (above right). Analysis of mouse pancreatic islets using a tissue classifier algorithm.

To quantify islet cell mass for a given mouse, stain and analyze at least **5 cryosections spaced approximately 200 µm apart**.

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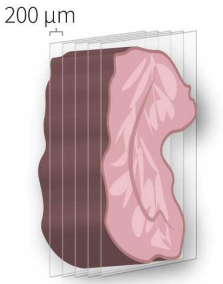

Each islet cell type is reported as a percentage of total alpha, beta, and delta cell area. Islet cell mass is calculated by multiplying alpha, beta, and/or delta cell area by pancreas weight.

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