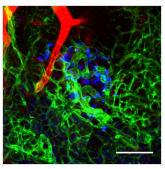

Aug 14, 2020

# Human Islet Microvasculature Immunofluorescence in Optically Cleared Samples

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1 *Works for me* dx.doi.org/10.17504/protocols.io.y3tfynn

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ABSTRACT

This protocol describes the immunostaining performed on five human control pancreas samples, cleared using a modified passive CLARITY (PACT) method according to our published methods. Islets were identified based on alpha-cells stained with glucagon while collagen-containing basement membranes in the extracellular matrix were stained using anti-collagen IV. Smooth muscle cells surrounding arteries and arterioles were identified using anti-SMA primary antibody directly conjugated with Cy3 fluorophore. An accompanying protocol describes using Vesselucida360 to contour islets and determine islet basement membrane and SMA densities and morphometric variables (https://dx.doi.org/10.17504/protocols.io.bjfzkjp6).

EXTERNAL LINK

https://www.protocols.io/view/human-pancreas-pact-optical-clearing-and-high-reso-9gbh3sn/materials

THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

Butterworth E, Dickerson WD, Vijay V, Weitzel K, Cooper J, Atkinson EW, Coleman JE, Otto KJ, Campbell-Thompson M. High resolution 3D Imaging of the Human Pancreas Neuro-Insular Network. J Vis Exp. 2018 Jan 29;(131). doi: 10.3791/56859.2018. PMID: 29443037.

ATTACHMENTS

jove-protocol-56859-high-resolution-3d-imaging-of-the-human-pancreas-neuro-insular-network.pdf

DOI

dx.doi.org/10.17504/protocols.io.y3tfynn

PROTOCOL CITATION

Martha Campbell Thompson, Elizabeth Butterworth Hosaka, Katelyn N Carty 2020. Human Islet Microvasculature Immunofluorescence in Optically Cleared Samples . **protocols.io** https://dx.doi.org/10.17504/protocols.io.y3tfynn

MANUSCRIPT CITATION please remember to cite the following publication along with this protocol

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KEYWORDS

human islet, glucagon, optical clearing, confocal microscopy, 3D, PACT, collagen IV, SMA, Vesselucida360, alpha-cells, pancreas, neuro-insular network, passive CLARITY



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IMAGE ATTRIBUTION

An islet is shown from case 6232 with all 3 fluorescent channels visible.

CREATED

Mar 12, 2019

LAST MODIFIED

Aug 14, 2020

PROTOCOL INTEGER ID

21331

GUIDELINES

This protocol is for PACT-cleared human pancreas samples, fixed with 4% paraformaldehyde for at minimum **48:00:00** , 4% agarose embedded and sectioned at 400 um or greater thickness. The Triton-X concentration in the antibody diluent was increased from 0.1% to 0.5% based on experiments that showed antibody penetration into the middle of the sample was improved without loss of signal.

MATERIALS

| NAME | CATALOG # | VENDOR |
|---|---|---|
| Glucagon (GCG) Mouse anti-human | ab10988 (Lot# GR290488-2) | Abcam |
| Goat anti-ms Dylight 405 hi-cross | 35500BID | Thermo Fisher Scientific |
| PBS Phosphate Buffered Saline 10X Solution | BP399-1 | Fisher Scientific |
| Goat normal serum | S-1000 | Vector Laboratories |
| Triton X-100 | T8787-100 | Sigma Aldrich |
| Collagen IV (Col14) Rabbit anti-human | ab6586 | Abcam |
| Smooth muscle actin- alpha (a-SMA) Mouse anti-human-Cy3 [clone 1A4] | C6198 | Sigma – Aldrich |
| Goat anti-rb AF488 hi cross | A-11034 | Thermo Fisher Scientific |

STEPS MATERIALS

| NAME | CATALOG # | VENDOR |
|---|---|---|
| PBS Phosphate Buffered Saline 10X Solution | BP399-1 | Fisher Scientific |
| Goat normal serum | S-1000 | Vector Laboratories |
| Triton X-100 | T8787-100 | Sigma Aldrich |

MATERIALS TEXT

**Vesselucida 360 v2020.1.1**
by MBF Bioscience



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**Vesselucida Explorer v2019.1.1**
by MBF Bioscience

EQUIPMENT

| NAME | CATALOG # | VENDOR |
|---|---|---|
| LSM 710 | LSM 710 | |

SAFETY WARNINGS

Follow all laboratory safety procedures when handling hazardous chemicals and materials.

DISCLAIMER:

> Different primary and secondary antibody lots may differ in affinities and should be independently optimized.

BEFORE STARTING

Ensure samples are cleared and the SDS is well-removed by extensive washing in PBS at **Room temperature** on a rocker plate.

1 For these samples, optical clearing was performed using 4% SDS at **60 °C** with rocking for up to 3 weeks until the samples reached transparency. Our original protocol used 8% SDS. We have subsequently tested both 4% and 8% SDS at **Room temperature** and **60 °C** . Different tissues should be tested independently for optimal results.

## Prepare the Antibodies

2 Make antibody dilution buffer (ABD): 1x PBS containing 2% normal goat serum (NGS) and 0.5% Triton-X 100.

PBS Phosphate Buffered Saline 10X Solution
by Fisher Scientific
Catalog #: BP399-1

Goat normal serum
by Vector Laboratories
Catalog #: S-1000



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Triton X-100
by Sigma Aldrich
Catalog #: T8787-100

3 Dilute the primary antibodies to 1:200 in ABD and prepare at least **1 mL** per sample.

Stain the Tissue

4 Incubate the samples with primary antibodies on a rocker plate for **48:00:00** 1-5 days at **Room temperature** .

5 Wash the tissue sections with 1xPBS for five times, minimum **01:00:00** at **Room temperature** .

PBS Phosphate Buffered Saline 10X Solution
by Fisher Scientific
Catalog #: BP399-1

6 Dilute the secondary antibodies to 1:500 and prepare at least **1 mL** per sample. Incubate for **48:00:00** or more on a rocker at **Room temperature** .

7 Wash the tissue with 1 x PBS 5 times **01:00:00** at **Room temperature** .

8 Equilibrate the samples in RIMS containing 0.5% sodium azide for at least **12:00:00** before imaging. Transfer to an imaging dish or chamber slide using RIMS as mounting media.



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Imaging

9 Perform confocal microscopy. Each microscope will require optimization of imaging parameters.

LSM 710
confocal laser microscope
**Zeiss LSM 710**

10 The following lasers were used for imaging each antigen in these images:

Track 1- 488- Collagen IV
Track 2- 405- GCG, 561- SMA

Results

11

Islet alpha-cells- GCG Stain - Specific Stain, Intensity Good, Background Low
Collagen IV- basement membranes- Specific Stain, Intensity Good, Background medium
SMA- smooth muscle cells surrounding vessels - Specific Stain, Intensity Good, Background Low

12 Analyze islet microvascular density by contouring the GCG+ area for islet volume followed by collagen IV and SMA morphometry using Vesselucida360.



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