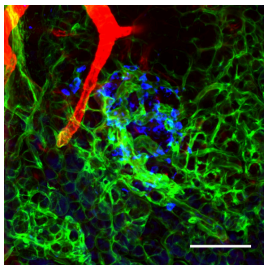
Aug 14, 2020

Human Islet Microvasculature Immunofluorescence in Optically Cleared Samples



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Protocol status: Working
We use this protocol and it's working

Created: March 12, 2019

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Protocol Integer ID: 21331

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

Disclaimer

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

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).

Attachments

PDF

jove-protocol-56859-...

786KB

Image Attribution

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

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

MATERIALS

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

STEP MATERIALS

- Triton X-100 **Merck MilliporeSigma (Sigma-Aldrich)** **Catalog #**T8787-100
- PBS Phosphate Buffered Saline 10X Solution **Fisher Scientific** **Catalog #**BP399-1

Software

NAME	DEVELOPER
Vesselucida 360	MBF Bioscience

Software

NAME	DEVELOPER
Vesselucida Explorer	MBF Bioscience

Protocol materials

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Safety warnings

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

Before start

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 **Fisher Scientific** **Catalog** #BP399-1

Goat normal serum **Vector Laboratories** **Catalog** #S-1000

Triton X-100 **Merck MilliporeSigma (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 **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.

Imaging

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

Equipment

LSM 710	NAME
confocal laser microscope	TYPE
Zeiss	BRAND
LSM 710	SKU
https://www.zeiss.com/microscopy/us/products/confocal-microscopes.html	LINK

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

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Expected result

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.