Human Pancreas Optical Clearing by iDISCO and VISIKOL

Martha Campbell Thompson[1], Malavika Nair[1]

[1]University of Florida

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1 Works for me

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SPARC

ABSTRACT

Optical clearing of tissues to improve deep tissue microscopy was described over a century ago and methods have improved considerably following publication of the CLARITY method from Karl Deisseroth in 2013. Improvements in organic and inorganic methods are constantly reported for multiple species and organs. Large volume imaging was needed for human pancreas studies due to known heterogeneity in normal islet sizes and proportions of endocrine cells. Lobularity in islet beta-cell loss and islet inflammation is also seen in patients with type 1 diabetes. The iDISCO protocol allows for rapid clearing, easy staining, and high resolution confocal imaging. Human pancreas samples were cleared using iDISCO as originally published. This protocol describes steps to clear 4mm x 6mm x 400um deep sections of 4% paraformaldehyde fixed human pancreas using iDISCO-like reagents purchased from Visikol.

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

GUIDELINES

As iDISCO and VISIKOL clearing utilizes organic reagents, imaging using a Zeiss Lightsheet Z1 is not feasible unless the sample is isolated in quartz glass to avoid damaging the objective. We have not attempted this and use a Zeiss 710 LSM microscope for imaging.

MATERIALS

NAME	CATALOG #	VENDOR
HISTO Kit	HSK-1	Visikol

MATERIALS TEXT

PBS buffer, methanol (MeOH), and other chemicals or reagents were purchased from Sigma and FisherScientific. Primary and secondary antibodies are listed in the JOVE publication. The iDISCO website contains a table of tested primary antibodies. We utilized cocktails of primary and secondary antibodies, combining primaries raised in mouse, rabbit, goat, and chicken and suitable secondary antibodies with AF or BV conjugates raised in donkey (ThermoFisher/Invitrogen, Jackson Immunoresearch).

Elizabeth Butterworth, Wesley Dickerson, Vindhya Vijay3 Kristina Weitzel, Julia Cooper, Eric W. Atkinson, Jason E. Coleman, Kevin J. Otto, Martha Campbell-Thompson (2018). High Resolution 3D Imaging of the Human Pancreas Neuro-insular Network. J Visual Experimentation.
http://10.3791/56859 (2018)

SAFETY WARNINGS

Handle all reagents according to your local EHS quidelines and as provided from the vendor.

Permeabilization 21h 30m

1 On a shaker, wash the fixed tissue 2x30 minutes in 1X PBS at room temperature. 1h

2 Dehydrate the tissue in increasing grades of methanol, 50%-80%-100% MeOH, for 1 hour each at room temperature. 3h

3 Place the tissue in 5% H_2O_2/20% DMSO/MeOH overnight on a shaker at room temperature. 12h

4 Wash tissue 2x1 hour in 20% DMSO/MeOH at room temperature. 2h

5 Wash tissue in decreasing grades of methanol, 100%- 80%-50% MeOH, for 1 hour each at room temperature. 3h

6 Place tissue in 1X PBS with 1% TritonX-100 for 30 minutes at room temperature. 30m

Labeling

7 Place the samples in Penetration Buffer for 36 hours at room temperature. 36h

8 Transfer the samples to Blocking Buffer and incubate for 36 hours at room temperature. 36h

9 Using the Antibody solution as a diluent, prepare the primary antibody cocktail and incubate the samples for 48 hours at room temperature. 48h

Primary and secondary antibodies are listed in the JOVE publication.

We utilized cocktails of primary and secondary antibodies, combining primaries raised in mouse, rabbit, goat, and chicken and suitable secondary antibodies raised in donkey (ThermoFisher, Jackson Immunoresearch).

10 Wash the samples in the Wash Buffer 5x30 minutes at room temperature. 2h 30m

11 Using the Antibody solution as a diluent, prepare the secondary antibody cocktail and incubate the samples for 48 hours at room temperature. 48h

12 Wash the samples in the Wash Buffer 5x30 minutes at room temperature. 2h 30m

Clearing 35h

13 Wash the tissue in increasing grades of methanol, 50%-80%-100% MeOH, for 1 hour each at room temperature. 3h

14 Incubate the samples in HISTO-1 solution for 16 hours at room temperature. 16h

15 Incubate the samples in HISTO-2 solution for 16 hours at room temperature. 16h

Imaging

16 Mount samples on glass slides in HISTO-2 with a #1.5 coverslip using silicon spacers or use glass chamber slides or small imaging dish.

17 Image using a confocal microscope or suitable Lightsheet.