Aug 30, 2023

Tissue clearing of human cardiac tissues using modified iDISCO+ protocol

DOI
dx.doi.org/10.17504/protocols.io.yxmvm3eo9l3p/v1

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Protocol Citation: Peter Hanna, Kalyanam Shivkumar, Shumpei Mori, Olujimi Ajijola 2023. Tissue clearing of human cardiac tissues using modified iDISCO+ protocol. **protocols.io https://dx.doi.org/10.17504/protocols.io.yxmvm3eo9l3p/v1**

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

Created: August 25, 2023

Last Modified: August 30, 2023

Protocol Integer ID: 86984

Keywords: tissue clearing of human cardiac tissue, using donor heart, imaging of the heart, human cardiac tissue, myocardial innervation of the human heart, donor heart, myocardial innervation, tissue clearing, human heart, sections of the heart, human transplantation, heart, imaging, tissue, confocal imaging, donor

Funders Acknowledgements:
NIH
Grant ID: OT2OD023848

Abstract

The purpose of this protocol is to evaluate the myocardial innervation of the human heart. Using donor hearts rejected for human transplantation, imaging of the heart was performed, and sections of the heart underwent tissue clearing and confocal imaging.

Troubleshooting

Tissue Fixation

1 Donor human hearts are obtained from the organ procurement organization and undergo perfusion fixation.

2 Suture three of the pulmonary veins and the inferior vena cava closed. One pulmonary vein and the superior vena cava are left patent as outflow of perfusate.

3 Cannulate aortic root and pulmonary artery with 24 Fr cannulas.

4 Perfuse 4% paraformaldehyde (PFA) via these cannulas at flow rates to distend the cardiac chambers for 24 hours at 4^{o}C.

5 Wash the heart to remove PFA with 0.01 mol/L phosphate buffered saline (PBS) perfusate for 30 minutes via the cannulas followed by three 0.01 mol/L PBS washes for 30 min.

6 Store the heart in 0.01 mol/L PBS/0.02% sodium azide at 4^{o}C until processing for immunohistochemistry or modified immunolabeling-enabled three-Dimensional Imaging of Solvent-Cleared Organs (iDISCO+) tissue clearing.

CT imaging, Photography and Dissections of Specimens

7 The heart is suspended in a container and scanned with a 64-multidetector-row computed tomographic scanner (SOMATOM Definition AS, Siemens Healthcare, Forchheim, Germany) with the following parameters: tube voltage, 120 kV; tube current, 800-900 mA; field of view, 100-200 mm; section thickness, 0.6 mm; an incremental interval, 0.3 mm; matrix size, 512∗512, and dose length product, 500-1200 mGy*cm. Postprocedural analysis is performed using commercially available software (Ziostation2; Ziosoft, Tokyo, Japan).

8 Specimens are dissected using either a #11 surgical scalpel or Metzenbaum scissors to generate adjacent samples intended for immunohistochemistry or tissue clearing using the modified immunolabeling-enabled three-Dimensional Imaging of Solvent-Cleared Organs (iDISCO+) protocol.

9 Hearts are photographed serially during dissection using a Nikon D850 DSLR camera with Nikon AF Micro-NIKKOR 200mm f/4D IF-ED lens.

Tissue Clearing using iDISCO+ Protocol

10 Fixed tissue undergo graded methanol (MeOH) dehydration (20, 40, 60 and 80% MeOH in diH2O [vol/vol]) for 1 hour each at room temperature (RT) with agitation.

11 Specimens are washed twice with 100% MeOH for 1 hour at RT and incubated in 66% dichloromethane/33% MeOH overnight at RT with agitation.

12 Tissues are then washed twice in 100% MeOH for 1 hour at RT, chilled at 4^{o}C and then bleached in 5% H2O2 in MeOH (vol/vol) overnight at 4^{o}C.

13 The tissues are rehydrated with a graded MeOH series and washed in 0.01 M PBS for 1 hour each at RT with agitation.

14 The tissues are washed in 0.01 M PBS with 0.2% Triton X-100 for 1 hour at RT.

15 Tissues are then stained by permeabilizing in 0.01 M PBS, 0.2% Triton X-100, 20% dimethyl sulfoxide (DMSO) and 0.3 M glycine and blocking in 0.01 M PBS with 0.2% Triton X-100, 10% DMSO and 5% donkey serum, each for 2 days at 37^{o}C with agitation.

16 Tissues are then incubated in primary antibodies rabbit anti-protein gene product 9.5 (PGP9.5; Abcam, ab108986, 1:200) and sheep anti-tyrosine hydroxylase (TH; Millipore, AB1542, 1:200) diluted in 0.01 M PBS with 0.2% Tween-20 and 10mg/ml heparin (PTwH) for 1 week at 37^{o}C with agitation to stain. PGP9.5 serves as a pan-neuronal marker while TH serves as a marker of sympathetic neurons. Primary antibodies are replenished mid-week.

17 Tissues are washed 4 to 5 times in PTwH overnight at RT and incubated with secondary antibodies donkey anti-rabbit Cy3 (Jackson ImmunoResearch, 711-165-152, 1:300) and donkey anti-sheep 647 (Jackson ImmunoResearch, 713-605-147, 1:300) for 1 week at 37^{o}C with agitation. Secondary antibodies are replenished mid-week.

18 Tissues are again washed in PTwH 4-5 times overnight at RT.

19 To clear, tissues are dehydrated with a graded MeOH series as above and incubated in 66% dichloromethane/33% MeOH for 3 hours at RT with agitation.

20 Tissues are washed twice in 100% dichloromethane for 15 minutes at RT and cleared, stored, and imaged in benzyl ether (Millipore Sigma, Catalog 108014; refractive index: 1.55).

Confocal Imaging of Cleared Tissues

21 Each iDISCO+-cleared tissue is placed in a chamber (SunJin Lab) filled with benzyl ether on a slide, and a coverslip applied.

22 Tilescan and Z stack images are obtained using a Zeiss LSM 880 confocal laser scanning microscope with a Fluar 5x/0.25 M27 Plan-Apochromat and 10x lens.

23 Images are obtained at a resolution of 1024 × 1024 using 488, 561, and 633 nm laser lines. Z-axis step size is commensurate with Nyquist sampling based on numerical aperture of the specified objective. Pinhole is set to 1 airy unit.

24 Stitched images are analyzed in Zeiss Zen Black SR and Bitplane Imaris 9.5.1 for 3-dimensional visualization.